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MANAGEMENT OF CERVICAL INTRAEPITHELIAL  
NEOPLASIA: IMPACT ON PRETERM BIRTH, POST-  
TREATMENT FOLLOW-UP AND HEALTH-RELATED  
QUALITY OF LIFE

**Annu Heinonen**

ACADEMIC DISSERTATION

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*To my family*

# ABSTRACT

Cervical cancer is the fourth most common cancer in women worldwide. It develops through precancerous stages that can either regress spontaneously or progress into cancer. Human papillomavirus (HPV) infection is a necessary but not alone sufficient factor in development of cervical cancer. Almost 200 HPV types have been identified and 13 of them are considered high-risk oncogenic types for cervical cancer. The prevalence of HPV is highest among young women and most infections are transient. Persistent infection may lead to precancerous lesions called cervical intraepithelial neoplasia (CIN).

Efficient screening programs with effective treatment of the precancerous lesions prevent 80 % of cervical cancer. Treatment of CIN aims to remove or destroy the lesion. This is most commonly done in an outpatient setting using a Loop Electrosurgical Excision Procedure (LEEP), in which an electrically charged loop wire is used to excise the lesion. The treatment is efficient but also has adverse effects on reproductive health. After treatment for CIN, women remain at a higher risk for recurrent CIN or cervical cancer for at least 20 years and need proper follow-up. The aim of this thesis was to provide extensive information on the treatment of CIN: its adverse reproductive effects, post-treatment follow-up and the psychological burden and Quality of Life.

LEEP has been associated with an increased risk for preterm birth. In our studies, we wanted to clarify this association further and assess the role of CIN itself. We studied the effect of severity of CIN and the time between LEEP and subsequent birth. In both studies regarding preterm birth, we formed large study cohorts using data from the Hospital Discharge Register (HDR) and the Medical Birth Register (MBR). In the first study the study population had 547 (7.2%) preterm singleton births compared to 30151 (4.6%) in the control population (OR 1.61, 95% CI 1.47–1.75). Repeated LEEP increased the risk almost threefold. The risk for preterm birth did not increase with increasing severity of CIN and the time between LEEP and subsequent delivery did not affect the risk for preterm birth.

In the second study, we included women diagnosed with CIN<sub>1</sub> and compared preterm birth rates among women with LEEP to those not treated. We also compared both groups to the general population (MBR) and each woman's deliveries before and after their CIN<sub>1</sub> diagnoses. The risk for preterm birth was increased among women treated with LEEP for CIN<sub>1</sub> compared to those in the MBR (OR 1.45, 95% CI 1.02–1.92). For CIN<sub>1</sub> patients not treated with LEEP, the risk for preterm birth was not increased compared to women in the MBR. The risk for preterm birth was also increased after CIN<sub>1</sub> diagnosis and LEEP (OR 1.47, 95% CI 1.05–2.06), whereas after only CIN<sub>1</sub> diagnoses without LEEP, the risk was not increased

(OR 0.90, 95% CI 0.71–1.13). There was no significant difference in the risk of preterm birth when comparing CIN1 patients with treatment to CIN1 patients without treatment (OR 1.31, 95% CI 0.94–1.83). Adjustments did not change the results. We also repeated analyses for primiparous and multiparous deliveries, but it did not change the results.

In Finland, colposcopy is still widely used in the follow-up of women after treatment of CIN. This consumes both time and resources. We wanted to study the role of colposcopy in post-treatment follow-up and assess different tests as predictors of treatment outcome. The study was part of a large prospective trial at the Helsinki University colposcopy unit. In this study the study population consisted of 419 women with LEEP for high-grade lesion and a follow-up visit six months after treatment. Overall, 2.4% of cases developed a recurrent disease. Colposcopy was a poor indicator of recurrence among these women (Sensitivity 0%, specificity 97%, PPV 0% and NPV 98%) and should be omitted in the primary follow-up. High-risk Human Papilloma virus (hrHPV) performed best with a sensitivity of 100%, a specificity of 85%, an NPV of 100% and a PPV of 12%.

The negative psychological effect of abnormal cytological findings and consequent examinations is widely acknowledged. We studied the general Health-Related Quality of Life (HRQoL) in women referred to colposcopy for abnormal cytology, and we further assessed different dimensions of their HRQoL and anxiety with questionnaires. The study comprised a prospective arm of patients from everyday clinical practice (n=238) and a retrospective arm where women treated eight years earlier were sent questionnaires (n=208). The general HRQoL score was not significantly different in patients than in the general population, meaning no effect on general HRQoL. However, patients scored significantly less on mental dimensions of the HRQoL tool. The severity of lesions did not affect results, but the negative psychological effect persisted for the 12-month follow-up period. In the retrospective arm, HRQoL was similar in patients and the general population except for a mildly lower score on the dimension of sexual activity.

In conclusion, our results are in line with previous studies confirming that LEEP is associated with an increased risk for preterm birth. Severity of the lesion or time between LEEP and subsequent birth had no effect on the increased risk. Colposcopy should be omitted in the follow-up of women after treatment for CIN, and hrHPV has been proven to be a reliable tool in detecting women at risk for recurrent disease. Our study confirms the psychological burden of abnormal cytology and referral to colposcopy. The general HRQoL was not affected in our study.

## FINNISH SUMMARY, TIIVISTELMÄ

Kohdunkaulansyöpä on maailmanlaajuisesti naisten neljänneksi yleisin syöpä. Sitä edeltää kohdunakaulansyövän esiasteet, jotka voivat parantua itsekseen tai edetä syöväksi. Ihmisen papillomavirus (HPV) on keskeinen mutta ei yksinään riittävä tekijä kohdunkaulansyövän synnyssä. HPV-tyyppejä tunnetaan yli 200 ja niistä 13 on todettu lisäävän merkittävästi riskiä sairastua kohdunkaulansyöpään. HPV-infektion esiintyvyys on yleisintä nuorilla naisilla ja suurin osa infektiosta on ohimeneviä. Pitkittynyt HPV infektio voi johtaa kohdunkaulansyövän esiaste-muutoksiin (CIN). Tupakointi on merkittävä kohdunkaulansyövän riskitekijä.

Tehokas seulpta yhdistettynä esiasteiden tehokkaaseen hoitoon estää 80% kohdunkaulansyövästä. Esiasteiden hoidossa poistetaan tai tuhoetaan muutosalue polikliinisesti sähköistettyä metallisilmukkaa käyttäen. Hoito on tehokas, mutta sillä on todettu haittoja lisääntymisterveydelle. Hoidon jälkeen esiasteen uusiutumisen riski on koholla ainakin 20 vuotta ja siksi potilaat tarvitsevat seuranta. Tämän väitöskirja-tutkimuksen tarkoitus oli tuottaa laaja-alaista tietoa kohdunkaulansyövän esiasteiden hoidosta ja sen vaikutuksesta lisääntymisterveyteen, seurannasta hoidon jälkeen ja vaikutuksesta elämänlaatuun.

LOOP-hoidon on osoitettu olevan yhteydessä suurentuneeseen ennenaikaisen synnytyksen riskiin. Halusimme tutkimuksissamme selvittää tätä yhteyttä ja arvioida esiasteen vaikeustason ja toimenpiteen ja synnytyksen välisen ajan vaikutusta. Molemmassa tutkimuksessa käytettiin syntymärekisterin ja hoitoilmoitusrekisterin tietoja tutkimuskohortin muodostamisessa. Ennenaikaisen synnytyksen riski oli kohonnut hoidon jälkeen. Ensimmäisessä tutkimuksessa tutkimusryhmällä oli 547 (7,2%) ennenaikaista synnytystä ja kontrolliryhmällä 30151 (4,6%) (OR 1.61 95%CI 1.47-1.75). Toistetut toimenpiteet suurensivat ennenaikaisen synnytyksen riskiä lähes kolminkertaiseksi. Muutoksen vaikeusaste tai aika toimenpiteen ja synnytyksen välillä ei vaikuttanut ennenaikaisen synnytyksen riskiin.

Toisessa tutkimuksessa tutkimusryhmän muodostivat naiset joilla oli CIN1 diagnoosi ja vertailimme LOOP-hoidettuja naisia ja seurannalla hoidettuja naisia keskenään. Vertasimme molempia ryhmiä myös naisiin syntymärekisterissä ja jokaisen naisen synnytyksiä ennen ja jälkeen CIN1 diagnoosin. Ennenaikaisen synnytyksen riski oli kohonnut naisilla joilla CIN1 oli hoidettu toimenpiteellä verrattuna tavalliseen väestöön syntymärekisterissä (OR 1.45, 95%CI 1.02-1.92). Sitä vastoin naisilla joilla CIN1 muutosta ei ollut hoidettu ennenaikaisen synnytyksen riski ei ollut koholla verrattuna yleiseen väestöön. Ennenaikaisen synnytyksen riski oli myös koholla CIN1 diagnoosin ja toimenpiteen jälkeen (OR 1.47, 95% CI 1.05-2.06), mutta ei pelkän CIN1 diagnoosin jälkeen (OR 0.90, 95% CI 0.71-1.13). Kun naisia joilla oli CIN1 diagnoosi ja LOOP-hoito verrattiin naisiin

joilla oli CIN1 diagnoosi ja ei LOOP- hoitoa ennenaikaisen synnytyksen riskissä ei ollut merkitsevää eroa (OR 1.31, 95 % CI 0.94-1.83).Kun analyyseissa huomioitiin tavanomaiset sekoittavat tekijät tai vain ensisynnytykset tai monisynnytykset, niin tulokset pysyivät samoina.

Suomessa kolposkopia on edelleen laajalti käytössä esiasteiden hoidon jälkeisessä seurannassa. Tämä vaatii aikaa ja resursseja. Halusimme tutkia kolposkopian roolia seurannassa ja arvioida eri testien kykyä arvioida hoidon onnistumista. Tutkimus oli osa laajempaa prospektiivista tutkimusta Helsingin Yliopistollisen sairaalan kolposkopiayksikössä. Tämän tutkimuksen tutkimusryhmä muodostui 419 naisesta, joilla oli vahva-asteinen esiaste hoidettu LOOP-hoidolla ja seurantakäynti kuusi kuukautta hoidon jälkeen. Yhteensä 2.4 % potilaista kehitti uusiutuvan taudin. Kolposkopia löysi huonosti naiset, jotka olivat riskissä solumuutoksen uusiutumiselle (herkkyys 0%, tarkkuus 97%, PPV 0% ja NPV 98%). Siten käytössä oleva rutiininomainen kolposkopia seurantakäynti voitaisiin poistaa käytännöstä. Korkean riski HPV-testi löysi puolestaan hyvin uusiutuman riskissä olevat naiset (herkkyys 100%, tarkkuus 85%, NPV 100% ja PPV 12%).

Poikkeavan solumuutoksen tiedetään vaikuttavan negatiivisesti henkiseen hyvinvointiin. Me tutkimme yleistä elämänlaatua ja ahdistusta naisilla, jotka oli lähetetty kolposkopiaan poikkeavan papa-muutoksen vuoksi. Elämänlaatua ja ahdistusta arvioitiin kyselykaavakkein. Tutkimuksessa oli prospektiivinen osa, jossa tutkimuskohortin muodostivat kolposkopiayksikön potilaat (n=238). Retrospektiivisessä osassa kahdeksan vuotta aiemmin hoidetuille naisille lähetettiin elämänlaatu kysely (n=208). Yleinen elämänlaatu ei eronnut potilailla verrattuna yleiseen väestöön. Kuitenkin psyykkisillä osa-alueilla potilaiden elämänlaatu oli alentunut. Solumuutosten vaikeusaste ei vaikuttanut elämänlaatuun, mutta pyykkisten osa-alueiden negatiiviset muutokset säilyivät 12 kuukautta. Retrospektiivisessä osassa ainoa ero potilaiden ja väestön välillä oli potilaiden lievästi alhaisempi seksuaalinen aktiivisuus.

Yhteenvetona tutkimustulostemme perusteella LOOP-hoito, toisin kuin pelkkä CIN1 diagnoosi, lisää ennenaikaisen synnytyksen riskiä ja tämä on linjassa aiempien tutkimustulosten kanssa. Muutoksen vaikeusaste tai aika hoidon ja synnytyksen välillä ei vaikuta ennenaikaisen synnytyksen riskiin. Kolposkopia voidaan jättää pois esiastemuutoksien hoidon jälkeisestä seurannasta. Korkean riskin HPV-testi on luotettava testi löytämään uusiutuvan taudin riskissä olevat naiset. Tutkimuksemme vahvistaa tietoa solumuutosten, esiasteiden ja kolposkopian aiheuttamasta psyykkisestä taakasta. Yleinen elämänlaatu ei huonontunut potilailla.

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# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

- I. **Heinonen A**, Gissler M, Riska A, Paavonen J, Tapper AM, Jakobsson M. Loop electrosurgical excision procedure and the risk for preterm delivery. *Obstet Gynecol.* 2013 May;121(5):1063-8.
- II. **Heinonen A**, Gissler M, Paavonen J, Tapper A-M, Jakobsson M. Risk of preterm birth in women with cervical intraepithelial neoplasia grade one: a population-based cohort study. *Acta Obstet Gynecol Scand.* 2018 Feb;97(2):135-41.
- III. **Heinonen A**, Jakobsson M, Kiviharju M, Virtanen S, Aro K, Kyrgiou M, Nieminen P, Kalliala I. Role of colposcopy after treatment for cervical intraepithelial neoplasia. *Cancers* Jun 2020;12(6):E1683
- IV. **Heinonen A**, Tapper AM, Leminen A, Sintonen H, Roine R.P. Health-related quality of life and perception of anxiety in women with abnormal cervical cytology referred for colposcopy: an observational study. *Eur J Obstet Gynecol Reprod Biol.* 2013 Jul;169(2):387-91.

# ABBREVIATIONS

15 D	15 D Health-related Quality of Life instrument
AGC-FN	Atypical glandular cells, favor neoplasia
AGC-NOS	Atypical glandular cells, not otherwise specified
AIS	Adenocarcinoma <i>in situ</i>
ASC-H	Atypical squamous cells, cannot exclude HSIL
ASC-US	Atypical squamous cells of undetermined significance
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN 1-3	Cervical intraepithelial neoplasia grades 1–3
CIN2+	Cervical intraepithelial lesion grade 2 and more severe
CIN3+	Cervical intraepithelial lesion grade 3 and more severe
CKC	Cold Knife Conization
DNA	Deoxyribonucleic acid
DS	Dual staining
HDR	Hospital discharge register
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HrHPV	High-risk human papilloma virus
HRQoL	Health-Related Quality of Life
HSIL	High-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
ICD10	International Classification of Diseases version 10
LBW	Low birth weight
LEEP	Loop electrosurgical excision procedure
LLEZT	Large loop excision of the transformation zone
LrHPV	Low-risk human papillomavirus
LSIL	Low-grade squamous intraepithelial lesion
MBR	Medical Birth Register
NPV	Negative predictive value
OR	Odds ratio
PPROM	Premature rupture of membranes
PPV	Positive predictive value
QoL	Quality of Life
RCI	Reid's Colposcopic Index
RR	Relative risk
SCC	Squamocellular cervical carcinoma
SCJ	Squamocolumnar junction
Sens	Sensitivity
SGA	Small for Gestational Age
Spec	Specificity
STAI	Welfare State-Trait Anxiety Inventory

THL	The Finnish Institute of Health and Welfare
TOC	Test of cure
TZ	Transformation zone
WHO	World Health Organization



# 1 INTRODUCTION

Of all cancers worldwide, 4.5% are related to Human papillomavirus (HPV), 8.6% in women and 0.8% in men. Cervical cancer is the fourth most common cancer of women worldwide, with an estimated 570,000 new cases in 2018 (Ferley *et al.*, 2018). Of these, 83% are HPV-attributable. Other HPV-attributable cancers include head and neck cancers and other anogenital cancers: vaginal, vulvar, anal and penile cancers (de Martel *et al.*, 2017). Thus, HPV infections and cervical cancer are important public health problems worldwide (IARC, 2007, 2012; Bruni *et al.*, 2010). The disease affects especially young fertile women (Winer *et al.*, 2003; Dunne *et al.*, 2007). Consequently, the effects on society are greater than those of many other malignant diseases, not overlooking the psychological burden recognized in women with HPV-related manifestations (Rogstad, 2002).

Cervical cancer is an optimal disease for screening. It has a long, treatable asymptomatic precancerous stage, offering a role for various screening techniques aiming to prevent cancer. Indeed, it has been demonstrated that 80% of cervical cancers can be prevented with efficient screening and treatment of precancerous lesions (Hakama and Räsänen-Virtanen, 1976; IARC, 2005; Arbyn *et al.*, 2010). At the same time, even after treatment, women remain at higher risk for recurrent disease for at least 20 years (Kalliala *et al.*, 2005), thus also requiring screening post-treatment.

Local surgical treatment of cervical intraepithelial neoplasia (CIN) is efficient in treating the precancerous lesions (Martin-Hirsch *et al.*, 2010). However, as per “*primum non nocere*,” treatment should do no harm. An association has been found between treatment for CIN and preterm birth (Kyrgiou *et al.*, 2016a). There is rising awareness about the need to individualize treatment. On the other hand, knowledge of the natural history of CIN is extending, and we know that a significant portion of lesions, even the high-grade ones, regress (Tainio *et al.*, 2018). More information is needed to elucidate the association between treatment of CIN and preterm birth.

The aim of this thesis was to provide widespread information on the treatment of CIN. The objective was to further clarify the association between preterm birth and the treatment of CIN with LEEP and to assess different follow-up strategies after treatment. Another objective was to study the Health-Related Quality of Life in women affected by HPV and CIN and procedures linked to them.

## 2 REVIEW OF THE LITERATURE

### 2.1 CERVICAL INTRAEPITHELIAL NEOPLASIA

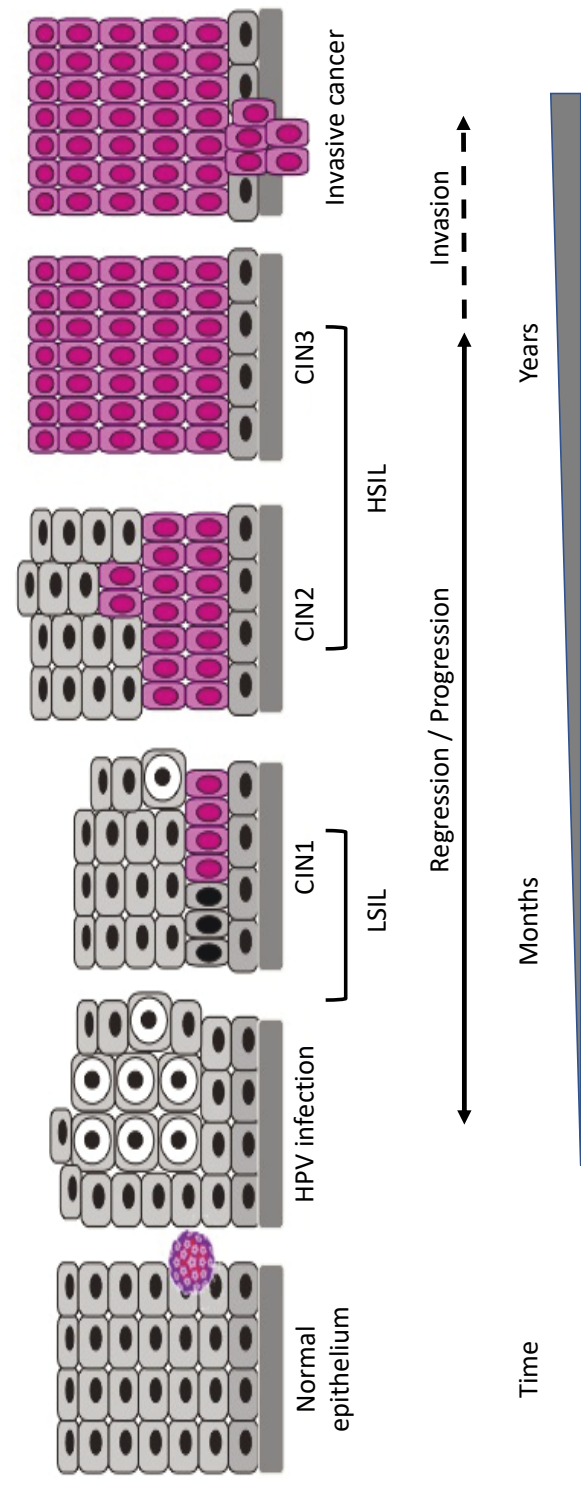
#### 2.1.1 DEFINITION

Cervical intraepithelial neoplasia (CIN) is a histopathological term for abnormal cell growth in the epithelium of the uterine cervix. The cervix is covered by two types of epithelia, the squamous epithelium on the ectocervix, which extends into the vagina, and the columnar epithelium on the endocervical canal, which connects to the uterine cavity and the endometrial lining. The border of these two epithelia is called the squamocolumnar junction (SCJ). Through a metaplastic process, columnar cells are replaced slowly by stratified squamous cells over years or decades. The area between the original SCJ and the new SCJ is called the transformation zone (TZ), and this is the most sensitive area for cellular abnormalities and CIN (Doorbar *et al.*, 2012). Cellular changes in CIN include disturbed cell growth and maturation, nuclear and cytoplasmic polymorphism and increased cellularity. The precancerous cells have many malignant features like cellular overcrowding, hyperchromatic nuclei and nuclear polymorphism. These features are restricted to the epithelium, whereas the basement membrane is not breached, and no infiltrative nor metastatic growth is present (Figure 1).

CIN is graded according to severity, therefore by the thickness of the affected epithelium. The old World Health Organization (WHO) classification released in 2003 graded the lesions as CIN1, CIN2 and CIN3. In CIN1, the lesion is restricted to the lowest third of the epithelium; in CIN2, up to two-thirds of the thickness of the epithelium is affected; and in CIN3, over two-thirds of the epithelium is affected, but the basement membrane is still intact (Figure 1). The WHO updated the classification in 2014, and CIN2 and CIN3 were combined into one high-grade entity called HSIL (High-grade squamous intraepithelial lesion). Histological low-grade squamous intraepithelial lesion (LSIL) comprises former CIN1 and HPV atypia (Table 1).

Abnormalities in columnar cells give rise to adenocarcinomas. The only known precursor for adenocarcinomas is adenocarcinoma *in situ* (AIS), but the natural history of the precursor to cancer in glandular cells is not known as well as in squamous cells (Krivak *et al.*, 2001).





**Figure 1.** Epithelial changes and progression of CIN 1–3 manifestations to cervical cancer. Adapted from Mitra *et al.*, *Microbiome* 2016.

**Table 1.** WHO nomenclature of histology for cervical lesions

	WHO 2003	WHO 2014
Cervical squamous		
	CIN1	LSIL
	CIN2	HSIL
	CIN3	HSIL
Cervical columnar		
	Glandular dysplasia	AIS
	AIS	AIS
	Microinvasive or invasive carcinoma	Invasive carcinoma
Vaginal		
	VAIN1	LSIL
	VAIN2	HSIL
	VAIN3	HSIL
Vulvar		
	VIN1	LSIL
	VIN2	HSIL
	VIN3	HSIL
	Differentiated-VIN (D-VIN)	D-VIN

### 2.1.2 ETIOLOGY

There is strong evidence that persistent or recurrent infection with human papillomavirus (HPV) is necessary for the development of cervical pre-invasive and invasive disease, but it is not sufficient on its own (zur Hausen, 1976; Bosch *et al.*, 1995a; Clifford *et al.*, 2003). HPV can be detected in practically all cervical cancer tissues (Walboomers *et al.*, 1999; Muñoz *et al.*, 2003).

HPVs are icosahedral double-stranded, non-enveloped deoxyribonucleic acid (DNA) viruses of the papillomaviridae family that have the ability to infect skin epithelium and oral or genital mucosa. Today, almost 200 HPV types have been identified and completely sequenced. (Bernard *et al.*, 2010; de Villiers, 2013; Bzhalava, Eklund and Dillner, 2015) Based on differences in their DNA sequence, HPVs are divided into five genera (Alpha, Beta, Gamma, Mu and Nu) (Bernard *et al.*, 2010). There are 40 subtypes of Alpha genera that can infect the anogenital area. These are further divided into two groups based on their oncogenic properties: low-risk HPVs (lrHPV), which are mostly associated with benign genital warts, and high-risk HPVs (hrHPV), which are the etiological agents for cervical cancer. There are 12 known hrHPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and one known as a

probable high-risk type: 68 (Doorbar *et al.*, 2015). The carcinogenic potential of the hrHPV types vary significantly. HPV 16 is the most carcinogenic type, and together with HPV 18 they account for 70 % of the squamous cell cancer cases worldwide. Along with types 31, 33, 35, 45, 52 and 58, they account for 90% of the cervical cancer cases. (de Sanjose *et al.*, 2010; Li *et al.*, 2011)

In addition to HPV infection, certain cofactors are necessary for progression from cervical HPV infection to cancer. Tobacco smoking increases the risk for cervical cancer twofold (Jensen *et al.*, 2012; Roura *et al.*, 2014) but seems to be associated only with squamous carcinoma (Berrington de González, Sweetland and Green, 2004). High parity, early onset of sexual activity and co-infection with Human immunodeficiency virus (HIV) have been identified as established cofactors (Berrington de González, Sweetland and Green, 2004; Castellsagué *et al.*, 2006; Singh *et al.*, 2009). The role of long-term oral contraceptive use has been long discussed and is believed to be a cofactor (Castle *et al.*, 2005; Muñoz *et al.*, 2006), although the results are conflicting (Jensen *et al.*, 2013). Even recent publications present contradictory results; an Australian study affirms oral hormonal contraceptives as a cofactor (Xu *et al.*, 2018), whereas Adhikari *et al.* found oral contraceptive use to have a protective effect against mild cervical cytological changes and CIN1 (Adhikari *et al.*, 2018). The use of an intrauterine device does not increase the risk for CIN or cervical cancer and may even reduce the risk of adenocarcinoma (Castellsagué *et al.*, 2006; Jensen *et al.*, 2013). Co-infection with other sexually transmitted diseases is a probable cofactor, and the most abundant evidence exists on chlamydia trachomatis infection (Anttila *et al.*, 2001; Muñoz *et al.*, 2006; Silva *et al.*, 2013).

### **2.1.3 EPIDEMIOLOGY AND NATURAL HISTORY OF HPV INFECTION**

HPV is a sexually transmitted infection that is usually acquired within a few years of sexual debut (Koutsky *et al.*, 1992; Woodman *et al.*, 2001; Stanley, 2010). The prevalence of HPV infection is high and highest among young women of 20-30 years of age (Dunne *et al.*, 2007) and the same age distribution has been found in incident HPV infections (Ho *et al.*, 1998; Rodriguez *et al.*, 2010a). In Finland the prevalence of HPV is 7,5 % among women in screening age (25-65 years) and the peak prevalence was 24,1% among women aged 24-29 years (Leinonen *et al.*, 2008). Furthermore a 2005 Finnish study found that a third of female university students were positive for HPV, and 84% of these infections were hrHPV types (Auvinen *et al.*, 2005). The most prevalent carcinogenic type in the screened population in Finland is HPV 16, followed by HPV 31 and HPV 52 (Leinonen *et al.*, 2013). Globally, the most prevalent HPV types are HPV 16, HPV 18, HPV 52, HPV 31 and HPV 58 (Bruni *et al.*, 2010).

However, most HPV infections are transient, and 70–90% of cases become undetectable within 1–2 years (Ho *et al.*, 1998; Moscicki *et al.*, 1998, 2012). The exact clearance time and definition of clearance varies across studies, but the median is about eight months (Rodriguez *et al.*, 2010a; Winer *et al.*, 2011). HrHPV infections clear in 12–18 months, whereas non-oncogenic infections clear faster, in 4–9 months. Prevalent infections take longer to clear than incident ones (Kjaer *et al.*, 2002; Koshiol *et al.*, 2006; Trottier *et al.*, 2008; Stanley, 2010). Infections of multiple HPV types have been associated with lower clearance rates (Ho *et al.*, 1998; Louvanto *et al.*, 2010).

HPV viral clearance is due to effective cell-mediated immunity shutting down viral gene expression in a CD4+ T cell-dominated Th1 (T helper cell 1) immune response (Monnier-Benoit *et al.*, 2006; Doorbar *et al.*, 2015). The host's failure to clear infection leads to persistent infection, which increases the probability of progression into high-grade and invasive cervical lesions, especially in hrHPV infections (Doorbar *et al.*, 2012, 2015). The risk of persistence seems to increase with age (Ho *et al.*, 1998; Rodriguez *et al.*, 2010a; Li *et al.*, 2019). HrHPV, specifically HPV 16 and HPV 33 infections, are more likely to persist followed by HPV 39, HPV 32 and HPV 58 (Bosch *et al.*, 1995b; Trottier *et al.*, 2008; Insinga *et al.*, 2011; Li *et al.*, 2019). In a recent Finnish study, the HPV distribution in HSIL lesions was found to be polarized by age: HPV 16/18 were most common in women under 30 years of age, whereas other hrHPV types were more common in HSIL lesions in women over 45 years (Aro *et al.*, 2019). As for detection rates, HPV detection seems to increase with disease severity: 50–70% positivity in CIN1/LSIL lesions, 85% in CIN2 lesions and 90–100% in CIN3 and invasive lesions (Bruni *et al.*, 2010; Guan *et al.*, 2012).

When HPV infection becomes undetectable, it is routinely described as viral clearance, but it may also represent immune control below detectable levels, i.e., viral latency (Gravitt and Winer, 2017). About 60% of HPV infections result in a detectable immune response (Carter *et al.*, 2000), but the ability to provide protection against re-infection is uncertain (Gravitt, 2012). Evidence is growing on HPV latency and re-activation of HPV infection (Maglennon, McIntosh and Doorbar, 2011; Liu *et al.*, 2014; Shew *et al.*, 2015; Winer *et al.*, 2016). An American study found that 30% of incident hrHPV infections were attributable to prior infection (with positive serology), whereas 40% were attributable to recent sexual behavior (with negative serology). The proportion of incident HPV infection attributable to recent sexual behavior decreased with age, and the writers stated that, among women with prior seropositivity, re-detection of the same hrHPV is likely due to intermittent detection of persistent infection or reactivation (Fu *et al.*, 2016).

#### 2.1.4 NATURAL HISTORY OF CIN

Cervical precancerous lesions are dynamic lesions that persist, regress or progress over time depending on host and lesion characteristics such as HPV type and cofactors. All changes in CIN development are reversible except the last stage leading to invasive lesions (Figure 1).

Low-grade lesions of the cervix, LSIL or CIN1 are generally only a manifestation of HPV infection; the spontaneous regression rate in adult women is up to 70–80% (Syrjänen *et al.*, 1992; Cox, Schiffman and Solomon, 2003) and in adolescent and young women under 22 years of age, the rate increases to 90% (Moscicki *et al.*, 2004). In an English prospective study, the progression rate for CIN1 lesions to CIN2+ (CIN2 or more severe) lesions was 12%, and the median time for progression was 25 months (Gurumurthy *et al.*, 2014).

In contrast, CIN3 is considered a true precursor for cervical cancer. The progression rate from CIN3 to invasive disease has been reported to be 15–39%, according to various studies (Hakama and Räsänen-Virtanen, 1976; Gustafsson and Adami, 1989; Mitchell *et al.*, 1996). A study from New Zealand reported a 20% risk of cervical cancer or vaginal vault within 10 years of untreated CIN3. One-third of women who had only suboptimal treatment for CIN3 progressed to cancer within 30 years and had a tenfold risk of cancer compared to women who had appropriate treatment for CIN3 (McCredie *et al.*, 2008; Mccredie *et al.*, 2010).

CIN2 lesions have a different natural history than CIN3. CIN2 comprises a heterogeneous group of lesions, including manifestations of HPV infections and true precursors (Carreon *et al.*, 2007). Multiple studies and a recent meta-analysis have shown that, in young women under 30 years of age, 60% of CIN2 lesions regress within two years, while only 11% progress to CIN3 or worse (Castle *et al.*, 2009; Moscicki *et al.*, 2010; Loopik *et al.*, 2016, 2019; Tainio *et al.*, 2018). New biomarkers to predict the progression are being researched widely. For example, DNA methylation has been shown to increase with CIN severity, and hence, it is suggested as a biomarker for progression (Lorincz *et al.*, 2013; Mirabello *et al.*, 2013; Kalantari *et al.*, 2014). In a recent first prospective study, methylation showed high potential as a prognostic marker for progression (Louvanto *et al.*, 2019). Further studies and development are still needed to make methylation a cost-effective marker for progression.

The development of cervical cancer is a long, multi-step process. The time between initial exposure to HPV infection and cervical cancer is thought to be 10–15 years. The time between HPV infection and CIN3 is thought to be shorter than between the first small CIN3 lesion and invasion (Ylitalo *et al.*, 2000; Winer *et al.*, 2005). Repeated hrHPV positivity is a risk for persistence, as are lesions of CIN3 or more severe (CIN3+). In an American study, the risk for CIN3+ within five years of an hrHPV-positive result was 7.6% (Katki *et al.*, 2011), and in a large Danish study, 25% of HPV 16-positive women developed CIN3+ lesions within 12 years (Kjaer *et al.*, 2010).

Prevalent infections are more likely to persist, and the risk of CIN3+ is highest with prevalent infections (Rodriguez *et al.*, 2010b). On the contrary, the risk of CIN3+ lesions after a negative hrHPV test has been reported to be very low, 0.17% (Katki *et al.*, 2011) and 0.27% (Dillner *et al.*, 2008).

## 2.2 PREVENTION OF CERVICAL CANCER

### 2.2.1 SCREENING

Secondary prevention of cervical cancer i.e. screening, aims to reduce the incidence and mortality of the cancer. The conventional screening method has been analysis of a cytological sample from the cervix, a Papanicolaou (Pap smear) smear (or test), introduced in the 1940s by Georgios Papanicolaou. The sample consists of three individual scraping samples collected from the vagina, cervix and endocervix, placed on a microscope glass slide and fixed immediately. Its efficacy has never been demonstrated in a randomized clinical setting, but it has been estimated that 80% of cervical cancer cases can be prevented in a well-organized screening setting (IARC, 2005; Arbyn *et al.*, 2010). The interpretation of the Pap smear is based on a subjective assessment of the pathologist (or cytotechnician), and the accuracy of the test is highly variable. The sensitivity in identifying CIN2 or worse (CIN2+) lesions has been reported worldwide to be around 52%, and the specificity 93–100% (Cuzick *et al.*, 2006; Castle *et al.*, 2011). In Finland, the cross-sectional sensitivity for CIN2+ lesions of conventional cytology has been reported to be as high as 83%, and the specificity 94% (Nieminen *et al.*, 2004). Conventional screening has been shown to be less effective in preventing mortality from cervical adenocarcinomas than squamocellular carcinomas (SCCs) (Nieminen, Kallio and Hakama, 1995; Sasieni, Castanon and Cuzick, 2009).

The variability in the sensitivity of the cytological evaluation gave rise to the interest in using the hrHPV test in primary screening. HrHPV has been shown to have superior sensitivity for detecting precancerous lesions and cancer (Ronco *et al.*, 2010). The specificity of the hrHPV test is roughly 3–10% lower than that of conventional cytology, but it increases with age (Cuzick *et al.*, 2006). In a Finnish study, Leinonen *et al.* found that hrHPV was more sensitive than conventional screening and also more specific in women over 35 years than conventional cytology (Leinonen *et al.*, 2009).

Municipalities in Finland are required by legislation to organize cervical cancer screenings for women between 30 and 60 years in five-year intervals. Most municipalities do so by inviting women to have a Pap smear. Municipalities can also decide to extend the screening to women aged 25–30 and 60–65. The city of Tampere in Finland changed to an HPV-based screening program in 2012, and the capital area followed at the beginning of 2019. Both the HPV test and the cytological sample are taken simultaneously,

but the cytology is only read if the HPV test is positive, and cytology serves as a triage test for the urgency of colposcopy. In 2015, the attendance rate in cervical screening was 69% in Finland. The rate of attendance has been decreasing, especially in the youngest screening group of women (aged 25–35) ([www.syoparekisteri.fi](http://www.syoparekisteri.fi)).

As screening shifts worldwide to primary hrHPV testing, triage strategies for hrHPV-positive women are needed. Repeating the hrHPV test one year after the first positive test to identify persistent infections is one method of triage. A prospective study conducted at Kaiser Permanente Hospital in California demonstrated that p16/Ki-67 dual staining (DS) of cytological samples is an effective triage test with equal immediate detection of precancerous lesions and substantially fewer referrals to colposcopy than in cytology alone (Wentzensen *et al.*, 2019). They also reported that the five-year risk of HSIL in DS-negative women was lower than in cytology-negative women (Clarke *et al.*, 2019). HrHPV 16/18 genotyping has also been suggested as a triage test for hrHPV-positive (and cytology-negative) women in deciding whether immediate referral to colposcopy is needed or if a repeated hrHPV test is an adequate approach (Schiffman *et al.*, 2015). Methylation and its role in triaging women with hrHPV is being studied widely and is found to be more sensitive and specific than HPV 16/18 genotyping (Lorincz *et al.*, 2016).

### 2.2.2 VACCINES

The first vaccine that was approved was a quadrivalent vaccine, *Gardasil*<sup>®</sup>, which targeted against HPV 6, HPV 11, HPV 16 and HPV 18, but it has now been replaced with a nonavalent vaccine, *Gardasil9*<sup>®</sup>, which is additionally targets against HPV 31, HPV 33, HPV 45, HPV 52 and HPV 58. The bivalent vaccine *Cervarix*<sup>®</sup> targets against HPV 16 and HPV 18. All three vaccines contain virus-like particles (VLP) (Roldão *et al.*, 2010).

All the vaccines have been reported to be efficient against HPV-related endpoints. In a large randomized multicenter trial, the quadrivalent vaccine was reported to prevent 98% of high-grade (CIN2+) lesions in young women with no previous HPV infection during a three-year follow-up period. The preventive effect of vaccination was 44% if accounting for women with previous HPV-infection (FUTURE II Study Group, 2007). A follow-up study of the FUTURE (Females United to Unilaterally Reduce Endo/Ectocervical Disease) group in the Nordic area was published in 2015, and they found that antibody titers for HPV 16, HPV 18, HPV 6 and HPV 11 were above the seropositive threshold limit nine years after vaccination (Nygård *et al.*, 2015). The randomized multicenter PATRICIA trial studied the bivalent vaccine and found that it prevents 93% of CIN2+ lesions during a three-year follow-up period (Paavonen *et al.*, 2009). The follow-up (four-year) results from the PATRICIA trial, which reported only CIN3+ and AIS lesions, found that the vaccine prevents all of these lesions (Lehtinen *et al.*, 2012). The end of the

PATRICIA trial's study analysis also found a significant cross-protection against four oncogenic nonvaccine HPV types: HPV 31, HPV 33, HPV 45 and HPV 51 (Wheeler *et al.*, 2012). A recent study showed that anti-HPV 16 and anti-HPV 18 antibody levels remain stable and above the natural infection level for up to 12 years after both the bivalent vaccine and the quadrivalent vaccine (Artemchuk *et al.*, 2019). The quadrivalent vaccine was replaced by the nonavalent vaccine; the two were compared in a randomized international trial, which found the nonavalent vaccine to be non-inferior to the quadrivalent vaccine (Joura *et al.*, 2015). Compared to the quadrivalent vaccine, the nonavalent vaccine has been reported to show high and sustained efficacy against HPV 31, HPV 33, HPV 45, HPV 52 and HPV 58 and a non-inferior efficacy against HPV 16, HPV 18, HPV 11 and HPV 6 (Huh *et al.*, 2017).

The girls-only vaccination strategy with a coverage of 70–90% has been reported to have a substantial herd effect against HPV 16 and HPV 6/11 (Cameron *et al.*, 2016; Kavanagh *et al.*, 2017; Drolet *et al.*, 2019). Globally, however, vaccination coverage is only moderate (Elfström, Dillner and Arnheim-Dahlström, 2015), and a gender-neutral strategy has been proposed to be more effective in moderate coverage areas (Brisson *et al.*, 2016). A Finnish community-randomized study assessed the bivalent vaccine efficacy, the herd effect and the overall protective effectiveness of a girl-only strategy versus a gender-neutral strategy. The study found that only the gender-neutral strategy provided a herd effect against HPV 18 and HPV 31 and an increased herd effect against HPV 33 and HPV 45. The overall protectiveness of HPV 31/33/35 were non-inferior to HPV 16/18, and the investigators stated that the gender-neutral approach can rapidly substitute lower vaccine efficacy against cross-protected HPV types (Lehtinen, Luostarinen, *et al.*, 2018; Lehtinen, Söderlund-Strand, *et al.*, 2018). Since the beginning of 2019, the Finnish Institute of Health and Welfare (THL) has recommended including boys in the vaccination program, but the execution of this recommendation is still halfway complete. For girls born in 2006, the overall coverage of the HPV vaccine in Finland in 2019 was 69.7%. There is much geographic variability in the coverage, with many areas reaching 80%. However, some areas, especially Ostrobothnia, have less than 50% coverage.

The safety of all HPV vaccines has been extensively studied. There have been no reports of an association between HPV vaccines and autoimmune, neurologic or venous thromboembolic adverse effects (Arnheim-Dahlström *et al.*, 2013; Lehtinen *et al.*, 2016; Arbyn *et al.*, 2018). A large Finnish nationwide report found no association of HPV vaccine with certain disease syndromes (POTS, postural, orthostatic tachycardia syndrome, Guillain-Barre syndrome, chronic fatigue syndrome, complex regional pain syndrome) commonly alleged as adverse events following immunization (Skufca *et al.*, 2018).



## 2.3 DIAGNOSIS OF CIN

The diagnosis of CIN is usually based on cytology and an hrHPV test supplemented and further defined by colposcopy-guided histological samples (biopsies). Colposcopy was first introduced in 1925 by Hans Hinselmann, and colposcopic examination was further developed by Walter Schiller, who introduced the use of Lugol's iodine solution in the examination. A colposcope is a binocular self-standing light microscope that magnifies the cervix 6–40 times the normal size, allowing the TZ and junction to be identified and inspected. Acetic acid (3–5%) solution is applied to the cervix to improve the visualization of abnormal areas; it causes the coagulation of superficial intracellular proteins and appears as reduced transparency of the cell, creating typical acetowhitening. Lugol's solution is still used today to further improve diagnostics. Colposcopy thus relies on the subjective assessment of the colposcopist, and it requires extensive training. The lesions are graded according to different grading scores: Reid's Colposcopic Index (RCI) or the Swede Score (Reid and Scalzi, 1985; Strander *et al.*, 2005) (Table 2). The scores evaluate the degree of acetowhitening, the presence of atypical vessels, and the shape and size of the lesion. The degree of acetowhitening is thought to be the most important in predicting CIN (Shaw, Sellors and Kaczorowski, 2003).

**Table 2.** Reid's Colposcopic Index and the Swede Score

			Score		
		Characteristic	0	1	2
Swede score	Reid's index (RCI)	Aceto uptake	Zero or transparent	Shady, milky (not transparent; not opaque)	Distinct, opaque white
		Margins/Surface	Diffuse	Sharp but irregular, jagged, 'geographical' satellites	Sharp and even, difference in surface level, including 'cuffing'
		Vessels	Fine, regular	Absent	Coarse or atypical
		Lesion size	<5mm	5-15mm or 2 quadrants	>15mm or 3-4 quadrants/ endocervically undefined
		Iodine staining	Brown	Faint or patchy yellow	Distinct yellow

Colposcopy has been shown in a meta-analysis to have a 96% sensitivity for squamous lesions but a poor specificity of 48% (Mitchell *et al.*, 1996). In an American study in which biopsies were taken from all four quadrants with an endocervical curettage rather than from the suspected areas identified in the colposcopy, the sensitivity for CIN2+ lesions was 57% (Pretorius *et al.*, 2004). In a review of the literature, the positive predictive value (PPV) of colposcopy to find CIN3+ lesions was 78% and for CIN2+ lesions 59% (Hopman, Kenemans and Helmerhorst, 1998). The predictive accuracy of colposcopy increases with both the knowledge of cytology and with increasing severity of the lesion (Kierkegaard *et al.*, 1994; Pretorius *et al.*, 2001). Moreover, in a study where still colposcopy images were assessed, the interobserver reproducibility was shown to be poor (Massad, Jeronimo and Schiffman, 2008). In an English study executed as part of a large randomized study (TOMBOLA), the risk for CIN3+ lesions after low-grade cytology and normal colposcopy was 4.6% during a follow-up time of three years (Cruikshank *et al.*, 2015). It has been suggested that, by taking multiple biopsies, the detection rate of histological high-grade lesions could be increased (Wentzensen *et al.*, 2015).

In Finland, women are referred to colposcopy according to the National Current Care Guidelines (Current Care Guidelines, 2019). The guidelines contain detailed instructions on cytology results and the need and urgency for colposcopy referral. Suspicion of carcinoma (cytological or clinical) indicates a colposcopy within seven days. The cytology indications for colposcopy within a month are HSIL, ASC-H (atypical squamous cells cannot rule out HSIL) and AGC-FN (atypical glandular cell, favor neoplasia). AGC-NOS (atypical glandular cell, not otherwise specified) indicates a colposcopy in two months. Colposcopy is indicated within 6 months after a repetitive (two to three times) ASC-US (atypical squamous cells of undetermined significance) within 12–24 months or a positive hrHPV test and repetitive ASC-US. Women over 30 with LSIL are sent to colposcopy within 6 months, but those under 30 years of age are sent according to the recommendation of the cytopathologist or if the follow-up smear at 6–12 months after LSIL is ASC-US or more severe. A repetitive positive hrHPV test (at 12-month intervals), even with normal cytology, is an indication for colposcopy. Other indications are repeated strong regenerative findings in cytology, post-coital bleedings and macroscopic condylomas of the cervix.

## **2.4 MANAGEMENT OF CIN**

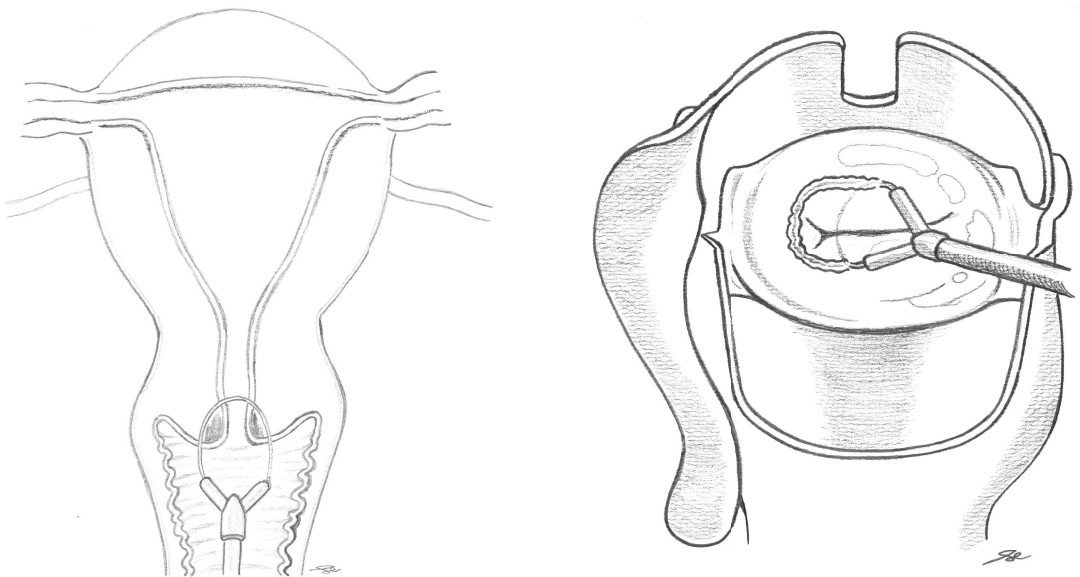
### **2.4.1 MANAGEMENT OPTIONS**

Treatment of CIN aims to destroy or remove the whole lesion or the whole TZ and the lesion within. Traditionally, prior to colposcopy, all lesions were

treated by cold knife conization (CKC), where the lesion is excised by a cold knife under general anesthesia in an operating room. Bleeding is a common perioperative problem. Successful treatment has been reported up to 93–96% (Tabor and Berget, 1990; Martin-Hirsch *et al.*, 2013).

Laser ablation is a destructive treatment that uses a laser beam to destroy the cells of TZ. The length of laser exposure controls the depth of the destruction. No histological sample is acquired from this technique, and therefore, histological samples beforehand are a requirement. The success rate for cervical treatment has been reported to be 95% (Jordan *et al.*, 1985; Martin-Hirsch *et al.*, 2013). Lasers are the treatment of choice in Finland only for vaginal lesions and for vulvar lesions where excision is not needed or possible. Needle excision (NETZ) is a procedure in which a straight diathermy wire is used to excise a lesion; it is rarely used nowadays, however.

Prendiville *et al.* introduced Loop conization (LEEP, LLETZ) in 1989 (Prendiville, Cullimore and Norman, 1989). In this procedure, tissue is excised using a small electrically charged hardwire loop for simultaneous cutting and electrocoagulation (Figure 2). It is performed in an outpatient setting under colposcopy control and under local anesthesia. Treatment success for LEEP has been reported to be 94–98% (Prendiville, Cullimore and Norman, 1989; Wright *et al.*, 1992; Martin-Hirsch *et al.*, 2013), and it has fast become the most popular mode of treatment due to fairly low cost, short procedure duration, good compliance and a fast and easy learning curve.



**Figure 2.** LEEP procedure. Figures by Sami Elamo

Excisional procedures are preferred because they make it possible to histologically examine the removed tissue and assess the completeness or success of the lesion's removal. However, no treatment has been proven superior to another in terms of success rates (Martin-Hirsch *et al.*, 2013). In Finland, LEEP is the golden standard treatment modality, and the Finnish Current Care guidelines recommend using ablative methods only on special occasions (Current Care Guidelines, 2019).

Treatment decisions should be based on the aggregate of the cytology, colposcopic impression and histology. The natural history of CIN lesions is important to consider when deciding on treatment options. The high probability of regression of CIN1 lesions (Cox, Schiffman and Solomon, 2003; Moscicki *et al.*, 2004; Gurusurthy *et al.*, 2014) and the poor reducibility of the CIN1 diagnosis should be also be considered (Stoler, 2001). Hence, the Finnish Current Care guidelines and the European and American guidelines recommend that LSIL (CIN1) lesions should only be treated if they progress or persist for 24 months (Jordan *et al.*, 2009; Massad *et al.*, 2013; Current Care Guidelines, 2019). It is recommended that HSIL/CIN3 lesions be treated due to their higher progression potential (McCredie *et al.*, 2008; Jordan *et al.*, 2009). However, the guidelines state that, among women  $\leq 31$  years of age, HSIL/CIN2 lesions limited to two-quarters of the cervix can be closely monitored for up to 24 months with colposcopy in 6-month intervals for regression, given that nearly 60% of the lesions will regress in this population (Tainio *et al.*, 2018). Glandular precursors (AIS) are also recommended to always be treated. Hysterectomy is recommended for women diagnosed with AIS who have no future pregnancy plans. During pregnancy, conization is only recommended if invasion cannot be ruled out, as progression during pregnancy is rare (Wu *et al.*, 2014; Hong *et al.*, 2019).

In general, the histology of the lesions should be known before treatment. However, immediate treatment, also called “select-and-treat” management, can be considered if referral cytology is HSIL and if colposcopic impression is high grade (Current Care Guidelines, 2019). It has been suggested that “see-and-treat” management, where a lesion is treated if it is clinically considered to be of high grade regardless of referral cytology, would result in overtreatment, especially with low-grade cytology referrals (Cárdenas-Turanzas *et al.*, 2005; TOMBOLA GROUP, 2009; Bosgraaf *et al.*, 2013). In “select-and-treat” management with both high-grade cytology and high-grade impression on colposcopy, the overtreatment probability is lower (Bosgraaf *et al.*, 2013). In a systematic review and meta-analysis and in other recent studies, the overtreatment percent was comparable to two-step management and is therefore a considerable management option (Ebisch *et al.*, 2016; Ciavattini *et al.*, 2019). Immediate treatment should always be performed if referral cytology is AGC-FN or if it is HSIL and colposcopy is

not satisfactory, meaning TZ is not visible (TZ3) (Current Care Guidelines, 2019).

#### **2.4.2 COMPLICATIONS RELATED TO TREATMENT OF CIN**

Cervical conization is usually a very well tolerated procedure, and short-term complications are not common. Of all treated women, 2–18% experience pain during treatment despite local anesthesia. Perioperative bleeding that disturbs the procedure occurs at 2–12% of treatments. Heavy postoperative or secondary bleeding and infections are even more uncommon (Gunasekera, Phipps and Lewis, 1990; Mitchell *et al.*, 1998; Dunn, Killoran and Wolf, 2004; Martin-Hirsch *et al.*, 2013). Prolonged vaginal discharge is common after cervical conization; the discharge is not necessarily infective, but due to secondary edema and the healing process of the cervix, and prophylactic antibiotics following excision are not recommended (Kietpeerakool *et al.*, 2017). Cervical stenosis occurs in 8–19% of women after LEEP (Martin-Hirsch *et al.*, 2013) and might be associated with the depth of the excised cone (Baldauf *et al.*, 1996). As part of a large prospective colposcopy study, the TOMBOLA trial, women were sent questionnaires about the after-effects of colposcopy and treatment for CIN. Bleeding was reported by 79%, and pain by 53% after colposcopy with biopsies and 87% and 63%, respectively, after LEEP (Sharp *et al.*, 2009).

#### **2.4.3 LONG-TERM OUTCOMES AFTER TREATMENT OF CIN**

The risk of any subsequent CIN is elevated for six years after treatment for CIN (Melnikow *et al.*, 2009; M Kocken *et al.*, 2011). Most recurrent cases appear within 12–24 months after the initial treatment (Ghaem-Maghami *et al.*, 2011a; M Kocken *et al.*, 2011). The risk also increases with the grade of the initial CIN (Melnikow *et al.*, 2009; Ghaem-Maghami *et al.*, 2011a). Involved margins at excision and persistent hrHPV infection after treatment predict treatment failure or recurrence (Arbyn *et al.*, 2017; Alder *et al.*, 2019).

The risk for cervical cancer after CIN treatment is elevated and stays elevated for at least 20–25 years after treatment (Kalliala *et al.*, 2005, 2020; Strander *et al.*, 2007; Rebolj *et al.*, 2012). The risk also increases with age. In a Swedish register-based study, the risk for cervical cancer after treatment for CIN3 was twofold compared to the general population, but the risk was fivefold for women aged 60–69 compared to women aged 30–39 at the time of the treatment (Strander *et al.*, 2007). The risk for vaginal and vulvar cancers was also increased after treatment for CIN (Kalliala *et al.*, 2005; Strander *et al.*, 2007). For glandular lesions, the risk of recurrence and cervical cancer is even higher than for squamous lesions, and they occur later, hence requiring a longer follow-up (Costa *et al.*, 2012).

## 2.5 TREATMENT OF CIN AND REPRODUCTIVE HEALTH

### 2.5.1 FERTILITY AND EARLY PREGNANCY

CIN itself and excisional treatment has not been found to be associated with infertility or first-trimester pregnancy loss (Bigrigg *et al.*, 1994; Cruickshank *et al.*, 1995; Spitzer *et al.*, 1995). In a Finnish retrospective cohort study, no difference in pregnancy incidence was found in women after conization when compared to the general population (Kalliala *et al.*, 2012). In a later retrospective cohort, they found no differences in miscarriages, termination of pregnancies or ectopic pregnancies in women before and after conization (Kalliala *et al.*, 2014). A meta-analysis and review and a Cochrane review concluded that excisional treatment does not affect fertility. They found no significant differences in the rate of first-trimester miscarriages between the general population and women treated for CIN (Kyrgiou *et al.*, 2014, 2015). However, a large recent register-based study from Norway found an association between first-trimester spontaneous miscarriage, LEEP and laser conization (Bjorge *et al.*, 2016). There is some evidence that a short interval (<12 months) between treatment and pregnancy might increase the risk of first-trimester miscarriage (Conner *et al.*, 2013; Ciavattini *et al.*, 2015). It has also been suggested that conization increases the risk for second-trimester miscarriage (Albrechtsen *et al.*, 2008), which was confirmed by two meta-analyses (Kyrgiou *et al.*, 2014; Kyrgiou, Mitra and Paraskevaidis, 2016).

### 2.5.2 PRETERM BIRTH

Due to well-functioning screening programs, precursors of cancer can be found and treated to prevent cancer. Hence, cancer incidence has been declining in recent decades in developed countries. Given that a large number of women treated for CIN are of reproductive age, there has been concern about the effect of the treatments on reproductive health. In the 1990s and early 2000, many studies were published on the association between all excisional treatments and preterm birth (birth <37 weeks of gestation), most reporting an association, though not always significant (Kristensen, Langhoff-Roos and Kristensen, 1993; Cruickshank *et al.*, 1995; Sadler *et al.*, 2004; Samson *et al.*, 2005). The same studies showed an increased risk for low birth weight (LBW) (birth weight <2500g) and premature rupture of the membranes (PPROM). The first meta-analysis and review on the matter was published in *Lancet* in 2006 by Kyrgiou *et al.*, and it concluded that there is an association between all excisional treatments and preterm birth. Additionally, LEEP was associated with LBW and PPRM. CKC was associated with an increased risk of cesarean section. Laser ablation was not associated with an increased risk for preterm birth (Kyrgiou *et al.*, 2006). Another meta-analysis was performed to assess the risk of serious adverse pregnancy outcomes. CKC was consistently associated

with severe and extremely severe preterm delivery (<32/34 and <28 weeks) and severe and extremely low birth weight (<2000g and <1500g). LEEP was not associated with severe adverse pregnancy outcomes (Arbyn *et al.*, 2008). Meta-analyses on conization and preterm birth are summarized in Table 3.

Large retrospective cohort studies using good quality data from the Nordic registries were published. These studies from Norway and Finland concluded that excisional treatment for CIN increases the risk for preterm birth, with no differences between treatment modalities (Jakobsson *et al.*, 2007; Albrechtsen *et al.*, 2008). Moreover, studies on only LEEP from Denmark and Finland reported an increase in the risk for preterm birth after the procedure (Jakobsson *et al.*, 2009; Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009b). A selection of studies assessing the association between LEEP and preterm birth are summarized in Table 4.

**Table 3.** Meta-analyses on conization and preterm birth

Author	Journal	n	Outcome	Findings	RR for PTB (95% CI)	Conclusion
Kyrgiou <i>et al.</i> 2006	Lancet	CKC 704 LEEP 1402 LC 562	Excision procedures on subsequent pregnancy outcomes	All excisional treatments associated with a small but statistically significant increase in pregnancy-related morbidity	CKC 2.59 (1.80–3.72) LEEP 1.70 (1.24–3.25) LC 1.71 (0.93–3.14)	Caution in treatment of young women with mild abnormalities
Arbyn <i>et al.</i> 2008	BMJ	CKC 343 LEEP 3392 LC 53	Serious adverse pregnancy outcomes (severe PTB<32 weeks)	CKC associated with severe preterm birth; LEEP not associated with severe or extreme preterm birth nor very low birth weight	CKC 2.78 (1.72–4.51) LEEP 1.20 (0.40–2.89) LC 3.33 (0.73–16.77)	Women of reproductive age should be informed of risk; Management should be tailored to minimize adverse effects
Bruinsma <i>et al.</i> 2011	BJOG	CKC 204 LEEP 10676 LC 195	Association between different excision techniques and preterm birth and effect of comparison group	All excision techniques associated with a significant increase in risk for preterm birth. Risk also increased in women with CIN and not treated	CKC 3.41 (2.38–488) LEEP 1.85 ( 1.59–2.15) LC 3.58 (1.93–6.61)	CIN itself increases risk for preterm birth; Suggests the comparison between CIN patients treated and not treated in future studies
Conner <i>et al.</i> 2014	Obstet Gynecol	LEEP 6589	Preterm birth after LEEP and whether increased risk is attributable to CIN or treatment	Risk of preterm birth is elevated for women with CIN regardless of treatment	LEEP 1.61 ( 1.35–1.92) LEEP vs. only dysplasia 1.01 (0.88–1.33)	Calls for larger studies with carefully selected comparison groups to clarify the role of CIN and treatment
Kyrgiou <i>et al.</i> 2014	BMJ	Miscarriages LEEP 686 CKC 448 Pregnancy rate LEEP 285	Fertility and early pregnancy outcomes	Excisional treatment does not affect fertility nor increase first-trimester miscarriages. Treatment might increase risk for second-trimester miscarriage	Miscarriage CKC 1.30 (0.92–1.83) LEEP 1.03 (077–1.36) Pregnancy rate LEEP 1.00 (0.67–1.48)	Fertility not affected by excisional treatment
Kyrgiou <i>et al.</i> 2016	BMJ	CKC 844 LEEP 21318 LC 672	Adverse obstetric outcomes in relation to excision cone depth	Women with CIN are at increased risk for preterm birth; Treatment further increases the risk; Risk increases with increasing cone depth	CKC 2.70 (2.14–3.40) LEEP 1.56 (1.36–1.79) LC 2.11 (1.24–3.57) LEEP 10–15mm 1.32 (1.02–1.72) LEEP >15 mm 3.16 (1.54–6.48)	In treating young women, efforts to optimize cone depth should be taken

LEEP, Loop electrosurgical excision procedure; CKC, cold knife conization; LC, laser conization; PTB, preterm birth; CIN, cervical intraepithelial neoplasia; RR, relative risk; 95% CI, 95% confidence interval



**Table 4.** Studies on the association of LEEP and preterm birth

Author	Journal	N (LEEP)	Outcome	Study design	RR/OR for PTB (95% CI)	Findings/Conclusion
Sadler <i>et al.</i> 2004	JAMA	278	PTB <37 weeks of gestation; First birth after LEEP	Retrospective cohort	RR 1.3 (0.9–1.9)	LEEP not associated with preterm birth but was associated with increased risk for PPROM; Recommend careful consideration in treatment of CIN in women of reproductive age
Samson <i>et al.</i> 2005	Obstet Gynecol	571	PTB <37 weeks of gestation	Retrospective cohort	OR 3.50 (1.90–6.95)	LEEP increases risk for PTB; Women should be informed of risk
Noehr <i>et al.</i> 2009	AJOG	8180	PTB <37 weeks of gestation PTB 28–31 weeks of gestation	Retrospective cohort	OR <37 weeks 2.07 (1.88–2.27) OR 28–32 weeks 3.28 (2.56–4.19)	LEEP associated with twofold increased risk of PTB; Risk even higher for severe PTB
Jakobsson <i>et al.</i> 2009	Obstet Gynecol	624	PTB <37 weeks of gestation	Retrospective cohort	RR 2.61 (2.02–3.20)	LEEP increased the risk for PTB; Unnecessary treatment should be avoided
Simoens <i>et al.</i> 2012	BJOG	53	PTB <37 weeks of gestation	Prospective cohort	OR 4.90 (1.72–13.96)	LEEP increased the risk for PTB; Suggest smaller cones when possible
Stout <i>et al.</i> 2015	BJOG	598	PTB <37 weeks of gestation	Retrospective cohort	RR 1.4 (1.1–1.8)	LEEP increased the risk for PTB; Suggests that presence of infection (chlamydia) after LEEP might increase the risk for PTB
Bjorge <i>et al.</i> 2016	Obstet Gynecol		PTB <37 weeks of gestation	Retrospective cohort	HR 1.5 (1.3–1.7)	LEEP increased the risk for PTB

PTB, Preterm birth; RR, relative risk; OR, Odds ratio; 95% CI, 95% confidence interval; HR, hazard ratio; LEEP, Loop electrosurgical excision procedure

The depth of the excised cone has been shown to have a role in the risk for preterm birth (Sadler *et al.*, 2004; Castanon *et al.*, 2014). A Danish register-based study found the risk for preterm birth to be directly associated with increasing cone depth, with an estimated 6% increase in the risk with each additional millimeter of depth (Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009a). This finding was confirmed in a large meta-analysis, concluding that the risk for preterm birth increases as depth of the excised cone increases, reporting a relative risk (RR) of 1.54 (1.09–2.18) for cones <10–12mm and 4.91 (2.06–11.68) for cones of 20mm or more in depth (Kyrgiou *et al.*, 2016a).

The role of conization in the risk of preterm birth has been debated. Studies disputing the association have been published, and the role of CIN itself has been suggested as the reason behind the increased risk (Shanbhag *et al.*, 2009; Castanon *et al.*, 2012; Reilly *et al.*, 2012; Conner *et al.*, 2014). An Australian register-based cohort study found that women only referred to colposcopy for cervical abnormalities had an increased risk for preterm birth, as did those treated for CIN, but after adjusting for confounding factors, the risk after treatment was not significant (Bruinsma *et al.*, 2006). In the retrospective cohort study by Reilly *et al.*, women referred for colposcopy were found to be at increased risk for preterm birth regardless of whether they received treatment. Treatment did not further increase this risk (Reilly *et al.*, 2012).

At the same time, a large meta-analysis and systematic review by Bruinsma *et al.* confirmed the association between excisional treatment and preterm birth, and they also stated that ablative treatments might also be associated with preterm birth (Bruinsma and Quinn, 2011). This association was confirmed by several other studies (Simoens *et al.*, 2012; Miller, Sakowicz and Grobman, 2015) as well as small (Danhof *et al.*, 2015) and large meta-analyses (Kyrgiou *et al.*, 2016a).

The question of why women with CIN are at increased risk of preterm birth is interesting. Rapidly evolving evidence indicating the vaginal microbiome might play a role in acquisition and persistence of HPV and progression of CIN, but also preterm birth. The normal healthy microbiome in the female reproductive tract is of low microbial diversity and dominated by a few species of *Lactobacillus* (Ravel *et al.*, 2011). Women with HPV infection have been found to have a more diverse vaginal microbiome than HPV-negative women (Lee *et al.*, 2013). In a very recent Norwegian cohort study, women with CIN were found to have a more diverse cervical microbiome than women with normal cytology, and LEEP appears to alter the cervical microbiome back toward lesser diversity (Wiik *et al.*, 2019).

## 2.6 FOLLOW-UP AFTER TREATMENT OF CIN

As stated before, the risk for recurrent CIN and cervical cancer is elevated after initial CIN treatment (Kalliala *et al.*, 2005; M Kocken *et al.*, 2011), and women need to be given a proper follow-up. The failure rate of excisional treatment, defined as the persistence or recurrence of HSIL lesions, has been reported to be 4–18%, with most new lesions occurring within two years of treatment (M Kocken *et al.*, 2011). Factors predicting the treatment failure have been extensively studied in trying to find a good indicator to identify women at increased risk for recurrent CIN or malignancy. Older age, large size and increasing severity of the lesion, involved cone margins, treatment modality and hrHPV persistence have been suggested as factors that predict recurrence (Soutter, Sasieni and Panoskaltsis, 2006; Ghaem-Maghami *et al.*, 2007; Strander *et al.*, 2007).

Positive or involved cone margins in the initial treatment have been shown to predict treatment failure (Dobbs *et al.*, 2000; Ghaem-Maghami *et al.*, 2007, 2011a). In a very recent register-based retrospective study from Sweden, women with involved margins at the initial cone were at increased risk for recurrent disease compared to women with free margins; however, the risk was not increased if the involved margins were only ectocervical (Alder *et al.*, 2019).

Persistence of hrHPV is another predictor of failure and has been widely studied in the follow-up after CIN treatment. It has been suggested that co-testing with cytology and hrHPV provided more assurance than either test alone (Bais *et al.*, 2009; Hormuzd A. Katki *et al.*, 2013). However, more recent studies find that hrHPV alone is a good negative predictor of recurrence and, hence, could be used alone as a test of cure (TOC) (Asciutto *et al.*, 2016; Bruhn, Andersen and Hariri, 2018). In a Danish study of women who tested negative for hrHPV six months after conization, the risk of CIN2+ lesions in the first five years after conization was similar to that of hrHPV-negative in the general population (Gosvig *et al.*, 2015).

A recent meta-analysis focusing especially on cone margins and hrHPV testing post-treatment stated that the risk of CIN2+ disease after treatment is significantly greater with involved excision margins. However, hrHPV testing post-treatment predicts treatment failure more accurately than margin status. They did pre- and post-testing in a plot using 20% probability as a threshold for referral to colposcopy and less than 2% as a threshold for returning to screening. Positive post-treatment hrHPV resulted in a 28.4% risk of treatment failure and negative hrHPV in a 0.8% risk. Positive resection margins, on the other hand, were associated with an average risk of post-treatment recurrence not reaching 20%, and negative resection margins were associated with a risk exceeding 2%, leaving all patients in the follow-up zone (Arbyn *et al.*, 2017).

HrHPV and margin status as predictors of treatment failure have been extensively studied, but the role of colposcopy has rarely been assessed.

Recent studies are scarce, and old studies present conflicting results (Flannelly *et al.*, 1997; Gardeil *et al.*, 1997; Baldauf, 1998). Many countries have abandoned colposcopy in the post-treatment follow-up based on the evidence of the good predictive value of hrHPV without evidence on the performance of colposcopy. The American guidelines recommend hrHPV at six months as a TOC irrespective of cone margin status. However they outline follow-up with colposcopy acceptable especially for women with positive cone margins status. (Perkins *et al.*, 2020). The European guidelines recommend a follow-up at 6, 12 and 24 months but with optional colposcopy, and they do not define the role of colposcopy (Jordan *et al.*, 2009). The UK NHS (National Health Services) guidelines recommend hrHPV 6 months after treatment as TOC but do not present specific evidence concerning colposcopy (NHS, 2020).

The Finnish Current Care Guidelines recommend hrHPV and cytology testing six months after treatment. Colposcopy is optional but is still widely used. After HSIL lesions, the follow-up is two years, along with recommended cervical screenings at five-year intervals. The follow-up, however, should last for at least 20 years. For AIS, the Current Care Guidelines recommend a follow-up colposcopy at 6 months, with a cytology and hrHPV and total follow-up time of 30 months before returning to screening.

## **2.7 HEALTH-RELATED QUALITY OF LIFE IN COLPOSCOPY PATIENTS**

### **2.7.1 DEFINITION AND MEASUREMENT OF HRQOL**

The WHO defines health as a state of complete physical, mental and social well-being and not merely as the absence of disease (WHO, 1958). This definition reflects the term health-related quality of life (HRQoL), which is a multidimensional concept comprising all of the aspects above: physical, mental, emotional and social. It can be defined as the well-being perceived by an individual or as the value of different dimensions used to measure QALY (quality-adjusted life-years) (Karimi and Brazier, 2016).

HRQoL can be measured with disease-specific or generic instruments. Generic instruments can compare different illnesses and their treatments, and there are several widely recognized generic instruments available. In the QALY context, the instrument must provide a single index score, with 1 representing optimal health and 0 equaling death. No HRQoL instrument has been proven to be superior when assessing HRQoL (Hawthorne, Richardson and Day, 2001).

Developed in the United Kingdom, the SF-36 is a widely used generic single-index score questionnaire that assesses HRQoL (Brazier, Roberts and Deverill, 2002). Many studies on HRQoL and on colposcopy patients use the

shorter version of the SF-36, the SF-6D, which measures the domains of physical functioning, role limitation, social functioning, pain, mental health and vitality.

Another widely used instrument is the EQ-5D, an HRQoL instrument developed as a European collaboration in an attempt to provide a simple tool to measure HRQoL. It measures the following domains: mobility, usual activities, self-care, pain or discomfort and anxiety or depression (Rabin and Charro, 2001).

The 15D is an HRQoL instrument developed in Finland in the 1980s. It is a generic single-index score instrument used to assess HRQoL (Sintonen, 2001). It contains 15 different dimensions: moving, seeing, hearing, breathing, sleeping, eating, speech (communication), excretion, usual activities, mental function, discomfort and symptoms, depression, distress, vitality and sexual activity.

HRQoL studies on gynecologic patients are scarce. A Finnish study found that the HRQoL of women with polycystic ovaries was significantly lower than the control population (Karjula *et al.*, 2020). Another Finnish study found that, when measured by 15D, gynecological malignancies have a negative effect on vitality, sexual activity and depression, but the effect is transient and the long-term HRQoL of gynecological cancer survivors is similar to that of the general female population (Pynnä *et al.*, 2019).

Anxiety can also be measured by generic instruments or disease-specific instruments. The State-Trait Anxiety Inventory (STAI) is a widely used questionnaire to assess anxiety (Spielberger, Gorsuch and Lushene, 1970). It consists of two 20-item scales: the Trait anxiety part, which measures proneness and the tendency to react to a situation perceived as threatening, and the State anxiety part, which measures momentary anxiety, or anxiety at a particular moment. The STAI measures anxiety levels on a scale of 20–80, and the scores are obtained by summing ratings of the individual questions.

## **2.7.2 HRQOL AND ANXIETY IN WOMEN WHO HAVE EXPERIENCED COLPOSCOPY**

It has long been recognized that abnormal cytology (Bell *et al.*, 1995; Gray *et al.*, 2006) and colposcopy both beforehand and during the procedure can provoke considerable stress and anxiety. Abnormal findings at the TZ in colposcopy are strong predictors of anxiety soon after colposcopy (Rogstad, 2002; Orbell *et al.*, 2004; Sharp *et al.*, 2011). The psychological impact has been suggested to be greater in menopausal women than in premenopausal women (Giannella *et al.*, 2013).

A Swedish longitudinal two-year study found that referral to colposcopy did not result in long-lasting depression or anxiety, but a small subgroup of women with pre-existing depression had higher depression rates at the two-year follow-up (Hellsten, Sjöström and Lindqvist, 2007).

Anxiety and depression have been widely studied in women with abnormal cytology, but studies on general HRQoL in these women are scarce. A study by Korfage *et al.* investigated the effect of cervical cancer screening in women with a normal cytology result; they documented the HRQoL and anxiety scores before screening, right after screening and after receiving the normal result. A considerable number of women reported unpleasant effects like feelings of shame, inconvenience and nervousness during sampling, vaginal bleeding and lower abdominal pain a few days after, but the general HRQoL was not affected (Korfage *et al.*, 2012).

Hellsten *et al.* studied health-related quality of life (HRQoL) using the SF-6D questionnaire. They did not find LEEP to affect the HRQoL. Referral for colposcopy had a long-lasting effect on mental health, but not on physical aspects of health (Hellsten, Sjöström and Lindqvist, 2009). Anogenital warts have been reported to have a significant negative effect on HRQoL, and the effect was the same for the first and recurrent episodes (Drolet *et al.*, 2011).

The see-and-treat or, more precisely, select-and-treat approach has been a growing trend in treating women with CIN. It has been reported that women managed with see-and-treat were less anxious than women with a two-step approach (Balasubramani *et al.*, 2007).

### 3 AIMS OF THE STUDY

The aim of this study was to provide widespread and extensive information on the treatment of CIN and its effect on the risk of preterm birth and on women's Health-Related Quality of Life. In more detail, the aims were:

1. To study the association between LEEP and preterm birth and further study the effect of the severities of CIN and of the time between LEEP and subsequent birth
2. To study the association between LEEP and preterm birth in more detail to assess the role of CIN itself and LEEP as a procedure
3. To evaluate the follow-up methods after treatment for CIN and provide information on the best follow-up strategy for the Finnish Current Care Guidelines
4. To study HRQoL and anxiety in women referred to colposcopy for an abnormal cytology result

## 4 MATERIAL AND METHODS

### 4.1 REGISTER-BASED STUDIES (STUDIES I AND II)

#### 4.1.1 REGISTERS

Studies I and II were both register-based cohort studies. The legislation in Finland ensures the collection of health information in various administrative and health registers. The validity and completeness of these registers are well established (Gissler and Shelley, 2002; Sund, 2012). Each citizen and permanent resident is given a unique encrypted personal identification number, which enables linkage among different registers. In these studies, we used the Hospital Discharge Register (HDR) and the Medical Birth Register (MBR). The National Institute of Health and Welfare (THL) maintain both.

The HDR collects information about all hospitalizations since 1967, all surgical procedures since 1994 and all out-patient visits since 1998. Surgical procedure codes are used to identify medical procedures (since 1997 based on the Finnish version Nordic Classification of Surgical Procedure). Diagnoses are registered for every patient contact, and for this, the latest version of the International Classification of Diseases version 10 is used (ICD-10).

The MBR is a nationwide register, and since 1987, it has collected baseline information on pregnant women and their health and data on all interventions during pregnancy and delivery. It also collects information on the newborn outcome for the first seven days after delivery.

#### 4.1.2 STUDY I

In this study our case women consisted of women in reproductive age (15-49) with a LEEP procedure done between 1997 and 2009 and their subsequent deliveries. We identified these women from the HDR by using the surgical procedure code LCD03, which has been used exclusively for LEEP since 1997. If a woman had an LCD03 code more than once at different time points she was considered to have had a repeated LEEP. Information about the severity of lesions or indications for LEEP were identified using the ICD10 codes as follows: R87.6 (abnormal cytology); A63.0 (condyloma accuminatum); N87.0 (CIN1); N87.1 (CIN2); N87.2 (CIN3); N87.9 (non-specific CIN); Do6.0, Do6.1, Do6.7 and Do6.9 (AIS); and C53.00–53.99 (SCC and adenocarcinoma of the cervix). The LEEP cases in which no corresponding diagnosis was found were considered non-specific. The most severe diagnosis either in biopsy or in the cone was recorded for each



woman. The group of women with HPV-related lesions milder than CIN1 (code A63.0) were classified into a non-CIN group. We then linked this information with the MDR information to collect data on the deliveries of the women after LEEP. We also assessed the time between LEEP and subsequent delivery.

Our case women consisted of 20 011 women with 25 101 LEEPs. Of these women, 5114 had subsequent singleton deliveries, the total being 7636 deliveries.

Our control population consisted of women in the MBR with no previous LEEP between 1997 and 2009. Our control group consisted of 430 975 women and their 658 179 singleton deliveries.

Preterm birth was defined as birth before 37 weeks of gestation. In Finland, gestational age is usually based on the first-trimester ultrasound. Extreme preterm birth (before 28 weeks of gestation) was analyzed separately. The risk of small for gestational age (SGA) according to national standards was also assessed. LBW (weight <2500 g) was also counted, as well as perinatal deaths (from 22 weeks of gestation until 1 week after birth).

The use of anonymous register data was authorized by the register-keeping organization, THL. Register authorities and a data-protecting ombudsman who reviewed the study before permission was given performed an ethical evaluation, as required by legislation.

#### **4.1.3 STUDY II**

In 2007, the Finnish Current Care Guidelines for cervical precursors were updated. The update recommended for the first time that women with CIN1 should be followed for 24 months instead of being given immediate treatment in order to identify and treat only persistent lesions. This allowed us to collect a unique study population of women with CIN and compare those who were treated and those who were not. Our goal was to study whether the increased risk for preterm birth was associated with CIN itself or with LEEP treatment.

We identified women aged 45 or younger with CIN1 between 1997 and 2011 from HDR and divided them into two groups according to whether they had had LEEP. To identify all their deliveries since 1987, we linked this information with MBR. To find women from the HDR, we used the ICD-10 code N87.0, and to find LEEP cases, we used the surgical procedure code LDC03. Women with previous diagnoses of more severe dysplasia or carcinoma were excluded. Our total study population consisted of 4759 women diagnosed with CIN1. These women had 4496 births before CIN1 diagnosis and 3017 after.

The cases in our study consisted of 2006 women with CIN1 diagnosis and LEEP treatment. In total, they had 797 deliveries, 334 of which were primiparous and 463 multiparous. Our control population consisted of 2753

women with diagnoses of CIN1 who had not been treated. They had a total of 2220 deliveries, of which 997 were primiparous and 1243 multiparous.

We compared these two groups and their risks for preterm birth. The risk for preterm birth was also assessed separately before and after LEEP treatment, including the deliveries before CIN1 diagnosis in the analysis. We also compared our study data with the general population in MBR. As secondary outcomes, SGA and LBW were assessed. Preterm birth was defined as birth before 37 weeks of gestation.

The ethical committee of Helsinki University Hospital approved the study (reference number 136/12/03/03/2012). THL approved the use of register data in scientific research as required by legislation.

## **4.2 PROSPECTIVE COHORT ON FOLLOW-UP AFTER TREATMENT (STUDY III)**

The aim of this study was to evaluate the performance and role of colposcopy in the follow-up after treatment for CIN. We wanted to compare colposcopy to hrHPV, cytology and cone margin status as a test of cure (TOC) post-treatment. This study was part of a large prospective cohort study (HELICOPTER study) in Helsinki University Hospital's Outpatient clinic, Colposcopy Unit. All patients referred to the unit between January 2014 and May 2016 were recruited. In this study, we included all women who had a LEEP and a follow-up visit at 6 months (n=491). In the main analysis the study population comprised of women with HSIL as worst histology and no repeated excisions. The women were further followed for 24 months according to Finnish Current Care Guidelines. The 12-month follow-up data were available for 70/419 women, the 18-month data for 45/419 and the 24-month data for 298/419 women. . Histological samples (punch biopsies) were taken at 303/419 of those 6-month visits. A flowchart is presented in Figure 3.

Recurrent disease was defined as histologically confirmed CIN2+. Subanalyses were conducted in which CIN1 was considered the threshold for recurrence.

Colposcopy was considered to be positive if the colposcopic assessment or impression was found to be HSIL/CIN2+ at the discretion of the individual colposcopist. The colposcopic assessment was available for 407/419 of the 6-month visits.

Cytology was considered positive if HSIL, ASC-H or FCG-FN were present. These data were available for 418/419 of the 6-month visits. Cytological and histological samples were reviewed by experienced gynecopathologists.

HrHPV samples were initially analyzed in Helsinki University Hospital's laboratory by Hybrid Capture II, a DNA test that detects HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. However, since April 2015, the

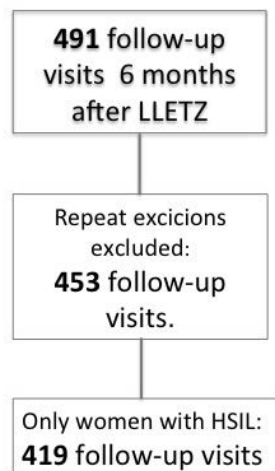
laboratory hrHPV testing was done with Aptima assay, an RNA test that detects HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The results of both tests are either positive or negative, and genotyping is not available. The hrHPV status was available for 407/419 visits at six months.

The excised cones were studied at Helsinki University Hospital's pathology department. The margin status was considered free (negative) or affected (positive), and the margin affected (endocervical, ectocervical or both) was noted. An unclear margin status was considered affected.

Results were also stratified according to smoking status at the time of LEEP.

The Ethical Committee of Helsinki University approved the study (reference number 130/13/03/03/2013), and written consent was collected from all participants.

**Figure 3.** Flowchart of Study III



## **4.3 PROSPECTIVE AND RETROSPECTIVE OBSERVATIONAL STUDY (STUDY IV)**

### **4.3.1 MATERIAL**

The aim of the study was to assess the HRQoL and anxiety in women referred to colposcopy because of an abnormal cytology result. This study consisted of two different arms, a prospective arm with 12 months follow-up and a retrospective arm where women treated years earlier were sent questionnaires to give insight into long-term effects.

The prospective part of the study was conducted at the Department of Obstetrics and Gynecology at Helsinki University Hospital. During 2007–2010, 500 women referred to colposcopy for an abnormal cytology result were sent HRQoL and anxiety questionnaires and an informed consent form with the appointment letter. 240 women answered the baseline questionnaires and they were sent follow-up questionnaires six and 12 months after the first visit. Non-respondents received one reminder. In addition, one question about seeking information and information sources was asked.

In the retrospective arm, 359 women who received treatment for CIN in the year 2000 were identified from the hospital records and were sent the HRQoL questionnaire. There were 208 respondents, 70 of which were treated for CIN1, 68 for CIN2 and 70 for CIN3.

The Ethical Committee of the University of Helsinki approved the study.

### **4.3.2 QUESTIONNAIRES**

HRQoL was measured with a generic, standardized, self-administered HRQoL instrument, 15D. It contains 15 different dimensions, and each is comprised of one question with a five-level answering option. The 15D instrument's scores are calculated from the state descriptive system by using a set of population-based preference or utility weights. The 15D compares favorably with other preference-based generic HRQoL instruments (Stavem, 1999; Hawthorne, Richardson and Day, 2001; Sintonen, 2001). The total index score that represents overall HRQoL has a 0–1 scale, where 1 equals optimal health and 0 equals death. A change of 0.015 in the score is considered clinically significant because, in general, a person can feel such a difference.

The patients' 15D results were compared with those of the general female population. The data for the comparison group for women over 30 years of age came from a representative sample from a national survey (Aromaa and Koskinen, 2004) and for women under 30 from another representative sample collected in 1995–1996 (Arinen *et al.*, no date). This combined general population sample was weighted to reflect the age distribution of the patients.

In the prospective arm, anxiety was assessed by the self-administered questionnaire STAI, and we used only the State part of the questionnaire. The STAI gives a score of 20–80 (low to high) to measure anxiety levels, and scores were obtained by summing the ratings of the individual questions. We created two groups of women representing high and low anxiety levels at the baseline: women who scored 35 or less at the baseline were considered to have low anxiety, and those scoring  $\geq 35$  were considered to have high anxiety levels.

#### **4.4 STATISTICAL ANALYSIS (STUDIES I–IV)**

In Study I, we calculated odd ratios (OR), 95% confidence intervals (CI) and number needed to harm (NNH) for preterm birth. The results were adjusted for maternal age, socioeconomic status, marital status, urbanism (urban, semi-rural and rural as defined by Statistics Finland) and time since LEEP. A second analysis was performed and results adjusted for all of the above and previous preterm births. Logistic regression analysis was used. We repeated the analyses including only first deliveries after LEEP (n=55 114) and primiparous deliveries after LEEP (n=53 355) to avoid the effect of clustering. Socioeconomic status was based on maternal occupation at time of birth and was classified according to Statistics Finland.

In Study II, we calculated OR and 95% CI. The results were adjusted for maternal age, marital status, socioeconomic status based on maternal occupation, urbanism and previous preterm birth. Logistic regression analysis was performed. Primiparous and multiparous deliveries were analyzed separately.

In Study III, all statistical analyses were done with SPSS statistical software version 24. Fisher's exact test was used to evaluate differences in proportions. The recurrence rate for HSIL/CIN2+ was assessed separately for hrHPV, colposcopy, cytology and margin status. The performance of these tests as tests of cure was assessed in terms of sensitivity (sens), specificity (spec), negative predictive value (NPV) and PPV to detect recurrence at follow-up.

In Study IV, the data were analyzed using SPSS for Windows statistical software version 17.0 (SPSS Inc., Chicago, IL, USA). The results are given as percentages or as mean (standard deviation SD). Student's paired sample t-test was used to analyze the differences at baseline and follow-up and between groups with one-way ANOVA followed by the Bonferroni post-hoc test or with an independent samples t-test. Values  $<0.05$  were considered statistically significant.

## 5 RESULTS

### 5.1 STUDIES I AND II

In Study I, the case women consisting of women with a LEEP procedure done between 1997 and 2009 were slightly older (mean age 30.8, SD 4.8) than the control population (mean age 30.0, SD 5.5), which consisted of women in the MBR without LEEP. The case women lived more often in urban areas than the control population, they were single more often, and the majority belonged to the socioeconomic class “other,” including students, stay-at-home mothers, etc. The number of previous births was approximately the same in both cases and controls. There were more smokers in the case women than in the general population, and 18% continued smoking even after their first trimester, in contrast to 11.6% of the control group.

In Study II, the case women included those with CIN1 diagnoses and LEEP. They were also slightly older (mean age 31.4) than the control population of women with CIN1 diagnoses but no LEEP (mean age 30.1). All women with diagnoses of CIN1 smoked more often than women in the general population. No differences between case women and the control population were found in socioeconomic status, marital status, urbanism, parity or gestational age.

#### 5.1.1 COMPARISON TO MBR

In Study I, the case women had 547 (7.2%) preterm singleton births in contrast to 30 151 (4.6%) in the control population (OR 1.61 95% CI 1.47–1.75). The overall preterm birth rate during the study period for singleton births was 4.6%. The risk for preterm birth did not increase with increasing severity of CIN, but for carcinoma *in situ* and cancer, the risk for preterm birth was somewhat higher. Repeated LEEP increased the risk for preterm birth almost threefold. Also, LEEP for non-CIN lesions (HPV-related lesions such as condylomatous atypia) increased the risk for preterm birth twofold (Table 5).

**Table 5.** The risk for preterm birth after LEEP, crude figures. The control population consisted of women in the Medical Birth Register without previous LEEP (n=25 101 women with preterm birth rate 4.45%).

Diagnoses	Treatments, n	Singleton births n	Preterm births, n (%)	OR (95% CI)
All	25 101	7636	547 (7.2)	1.61 (1.47–1.75)
Non-CIN	945	414	37 (8.9)	2.04 (1.46–2.87)
CIN grade unknown	104	24	1 (4.2)	0.91 (0.12–6.71)
CIN1	3686	1372	88 (6.4)	1.43 (1.15–1.77)
CIN2	4730	1549	88 (5.7)	1.25 (1.01–1.56)
CIN3	2559	808	51 (6.3)	1.40 (1.06–1.86)
Ca <i>in situ</i> or cancer	908	299	25 (10.9)	2.55 (1.68–3.87)
Repeated LEEP	4275	870	103 (11.8)	2.80 (2.28–3.44)

CIN, cervical intraepithelial neoplasia; OR, Odds ratio; 95% CI, 95% confidence interval; Ca *in situ*, carcinoma *in situ*; LEEP, loop electrosurgical excision procedure.

Adjustment for maternal age, urbanism, marital status and socioeconomic status did not change these results. Adjusting for previous preterm births did not change the results either. The time interval between LEEP and subsequent birth was also analyzed, and it had no effect on preterm birth (OR 0.98, 95% CI 0.96–1.00). Subanalyses showed that first births after LEEP and primiparous births after LEEP had similar risk scores (Table 6).

**Table 6.** LEEP and risk for preterm birth, adjusted results

	All deliveries OR (95% CI)	First deliveries after LEEP OR (95% CI)	Primiparous deliveries after LEEP OR (95% CI)
LEEP *	1.65 (1.47–1.85)	1.63 (1.34–1.98)	1.61 (1.34–1.93)
LEEP**	-	1.66 (1.37–2.02)	-
Repeated LEEP	2.77 (2.23–3.45)	2.52 (1.74–3.64)	2.59 (1.91–3.50)
Time interval since LEEP	0.98 (0.96–1.00)	0.99 (0.96–1.05)	1.00 (0.97–1.03)

\*Adjusted for maternal age, socioeconomic status, marital status, living environment and time since procedure.

\*\* Adjusted for all the above and previous preterm deliveries.

PTB, preterm birth; LEEP, Loop electrosurgical excision procedure; OR, Odds ratio; 95% CI, 95% confidence interval.

The risk for having an LBW infant was increased (OR 1.50, 95% CI 1.30–2.16). The risk for SGA (OR 0.27, 95% CI 0.70–1.31), extreme preterm birth (OR 1.27, 95% CI 0.75–2.16) or perinatal death (OR 1.25, 95% CI 0.76–1.89) were not increased.

In Study II, women diagnosed with CIN1 were compared to women in the MBR and were analyzed for the risk for preterm birth. The risk for preterm birth was increased for women treated with LEEP for CIN1 compared to those in the MBR (OR 1.45, 95% CI 1.02–1.92). Contrarily, for CIN1 patients not treated with LEEP, the risk for preterm birth was not increased compared to women in the MBR. The same risk pattern was found when analyzing multiparous births separately. However, when analyzing only primiparous births after LEEP, there was no increased risk for either group compared to women in the MBR.

The risk for LBW was increased for CIN1 patients with LEEP in multiparous births when compared to the MBR data. This risk was not observed among CIN1 patients without LEEP treatment. The risk for SGA was not increased for either group compared to the MBR data.

### **5.1.2 INTERNAL COMPARISON AND DISEASE-SPECIFIC COMPARISON**

In Study II, women with CIN1 and LEEP had 54 (6.7%) singleton preterm births in contrast to 116 (5.2%) in women with CIN1 without LEEP; this difference was not significant (OR 1.31, 95% CI 0.94–1.83). Adjustment did not change the results. Sub-analyses of primiparous (OR 1.12, 95% CI 0.69–1.83) and multiparous (OR 1.29, 95% CI 0.93–2.37) births had similar results.

We used an internal comparison and separately analyzed the risk for preterm birth before and after diagnoses of CIN1. The risk for preterm birth was increased after CIN1 diagnosis and LEEP (OR 1.47, 95% CI 1.05–2.06), whereas after only CIN1 diagnosis without LEEP, the risk was not increased (OR 0.90, 95% CI 0.71–1.13) (Table 7). In multiparous women, we detected the same increase in risk for preterm births after CIN1 and LEEP, but not for CIN1 only. However, the risk was not increased in either group when analyzing only primiparous women.



**Table 7.** Risk for preterm birth before and after CIN1 diagnosis

	Before		After		OR (95% CI)
	Full-term n	Preterm* n (%)	Full-term n	Preterm* n (%)	
No LEEP	2635	163 (5.8)	2104	116 (5.2)	0.90 (0.71–1.13)
LEEP	1620	78 (4.6)	747	64 (6.7)	1.47 (1.05–2.06)

LEEP, loop electrosurgical excision procedure

\* delivery before 37 weeks of gestation

## 5.2 STUDY III

In Study III, the mean age of the study population at the time of treatment was 35.1 years (20.1–76.6, SD 9.3). The indication for LEEP was persistent CIN1 in 1.7% of the cases, CIN2 in 40%, CIN3 in 29.6% and AIS for 0.2%. Two women had both CIN3 and AIS in biopsies (0.4%). In 105 cases (25.3%), immediate treatment was done due to type three TZ or suspicion of high-grade disease (select-and-treat).

In follow-up punch biopsies were taken at 303/419 (72.3%) of the 6 months visit. At 24 months 121/419 (28.9%) women were lost to follow-up.

Overall, 10 (2.4%) patients developed recurrent disease, 5 of which were diagnosed at 6 months and 5 at 12 months after LEEP.

Colposcopy was considered positive at 11/407 (2.7%) of the 6-month visits, but none of these women developed recurrent disease. Colposcopy was considered negative for 396/407 women (97.3%) and 9/396 (2.3%) had a recurrence. Of the recurrent cases, five were diagnosed at 6 months and four at 12 months. For one of the recurrent cases, the colposcopy assessment was not available.

At six months, cytology was positive for 6/418 (1.4%) of patients, and four 4/6 (57.2%) recurrent cases were found in this group, all of them at 6 months. In the group with normal cytology 412/418 (98.6%), six 6/412 (1.5%) patients developed recurrence, one at 6 months and five at 12 months.

Altogether, 82.3% (335/407) of patients tested negative for hrHPV at six months, and none developed recurrent disease during the follow-up. HrHPV was positive at 72/407 (17.7%) of the six-month visits, and 9/72 (12.5%) of those developed a recurrent disease, four at 6 months and five at 12 months. For one recurrent case the hrHPV status at 6 months was not known.

The cone resection margins were affected in 80 of the 419 LEEPs (19.1%). Recurrent disease was detected in six of these cases (6/80, 7.5%) three at 6 months and three at 12 months. For 339/419 (80.9%), the margins were free, and four 4/339 (1.2%) recurrent cases were diagnosed in this group, two at 6 months and two at 12 months.

When analyzing all women (n=491), regardless whether re-operation was done or of the histopathological grade of the lesion, margins were affected for 123/491 (25.0%) women. A re-operation was performed for positive resection margins in 38/123 (26.8%) and in only 10/38 (26.3%) of re-operation cones HSIL was still present and for 11/38 (28.9%) any dysplasia was present. For those patients with persistent dysplasia in re-operation cone, endocervical margins were most often originally affected (8/11).

Margins affected		6 months n/N (%)*	12 months n/N (%)	18 months n/N (%)	24 months n/N (%)	All cumulative n/N (%)
<b>Any margin</b>						
No re-LEEP, only HSIL (n=419)	Free (n=339)	2/339 (0.6)	2/51 (3.9)	0/28 (0.0)	0/236 (0.0)	4/339 (1.2)
	Affected (n=80)	3/80 (3.8)	3/19 (15.8)	0/15 (0.0)	0/47 (0.0)	6/80 (7.5)
All LEEP (n=491)	Free (n=368)	2/368 (0.5)	2/56 (3.6)	0/36 (0.0)	0/249 (0.0)	4/368 (1.1)
	Affected (n=123)	3/123 (2.4)	3/26 (11.5)	0/23 (0.0)	0/74 (0.0)	6/123 (4.9)
No re-LEEP	Affected (n=85)	2/85 (2.4)	3/20 (15)	0/16 (0.0)	0/469(0.0)	5/85 (5.8)
Re-LEEP	Affected (n=38)	1/38 (2.6)	0/6 (0.0)	0/7 (0.0)	0/25 (0.0)	1/38 (2.6)

**Table 8.** Recurrence of HSIL according to margin status

LEEP loop electrosurgical excision procedure. HSIL high grade cervical intraepithelial lesion.

The diagnostic accuracy of the different tests is presented in Table 9. HrHPV performed best with a sensitivity of 100%, a specificity of 85%, an NPV of 100% and PPV of 12%. Colposcopy performed poorly at six months (Sensitivity 0%, specificity 97%, PPV 0% and NPV 98%). Cytology gave a sensitivity of 40%, a specificity of 99%, PPV of 67% and NPV of 99%. The margin status of the first cone as a predictor of treatment outcome gave a sensitivity of 60%, a specificity of 82%, PPV of 8% and NPV of 99%.

Cigarette smoking did not affect the recurrence rates. Three recurrent cases were detected among smokers (3/143 2.1%) and six among non-smokers (6/250, 2.4%).

**Table 9.** Comparison of different tests of cure at 6 months and margin status at LEEP to predict recurrence of HSIL in 24-month follow-up

	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Cytology	0.40 (0.17-0.69)	0.99 (0.98-1.00)	0.67 (0.30-0.94)	0.99 (0.97-0.99)
Colposcopy	0.00 (0.00-0.30)	0.97 (0.95-0.98)	0.00 (0.00-0.24)	0.98 (0.96-0.99)
HrHPV	1.00 (0.70-1.00)	0.85 (0.81-0.88)	0.12 (0.07-0.22)	1.00 (0.99-1.00)
Margins	0.60 (0.31-0.83)	0.82 (0.78-0.85)	0.08 (0.03-0.15)	0.99 (0.97-1.00)

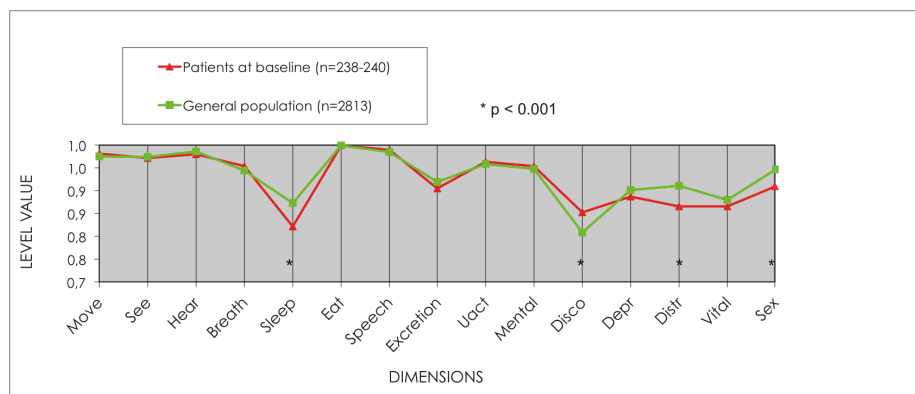
PPV, positive predictive value, NPV, negative predictive value CI Confidence interval

## 5.3 STUDY IV

### 5.3.1 HRQOL

The mean age of patients in the prospective part was 39 (SD 12, range 17–68) years. Complete 15D data were available for 48% of women at baseline (n=238), for 41% at 6 months (n=201) and for 36% at 12 months (n=181).

At baseline, the general 15D score was not significantly different in patients (0.933) compared to the general population (0.939). However, on the dimensions of sleeping, sexual activity and distress, the patients scored lower than the general population ( $p < 0.001$ ). On the other hand, the general population scored lower on the dimension of symptoms and discomfort ( $p < 0.01$ ) (Figure 4).



**Figure 4.** Mean 15D profiles at baseline for patients compared to the age-standardized general population.

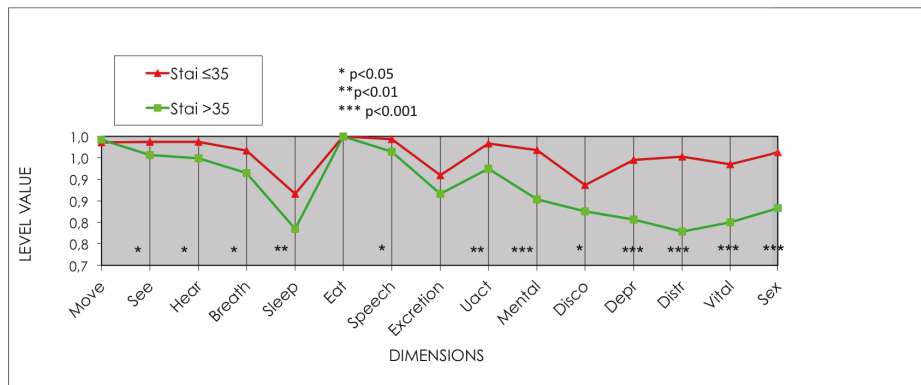
When comparing baseline results with results at 12 months for those having answered at all three time points, the 15D general score was just slightly lower at 12 months than at baseline ( $p=0.049$ ). The dimensions of mental function and usual activities were significantly lower at 12 months than at baseline ( $p<0.05$ ). The severity of the referral cytology did not affect HRQoL. When divided into two groups according to whether or not dysplasia was present in histological samples, the only difference was at 12 months, at which time the dysplasia group scored lower on dimensions of vitality and distress ( $p<0.05$ ).

The mean age in the retrospective arm was 43 (SD 12, range 26–79). Complete 15D data were available for 58% ( $n=208$ ). The mean general 15D (0.934) score did not differ significantly from the general population (0.932), which scored lower on the dimension of symptoms and discomfort ( $p<0.001$ ). According to severity of original histological findings, patients were placed into three groups: mild, moderate and severe. The only difference was found between the mild and severe groups in the dimension of sexual activity ( $p<0.05$ ).

### 5.3.2 ANXIETY

The STAI score was available at baseline for 45% of women ( $n=226$ ), for 35% ( $n=175$ ) at 6 months and for 33% ( $n=166$ ) at 12 months. The STAI scores were 20–67 at baseline (mean 34), 20–68 at 6 months (mean 34) and 20–79 at 12 months (mean 34). Complete STAI questionnaires were available for 133 women at all three time points, and no significant changes in mean STAI scores were found over time.

Women with high anxiety scores ( $>35$ ) at baseline showed a significantly lower 15D general score (0.901) than those with low anxiety levels at baseline (0.967) ( $p<0.001$ ). There was a significant difference at baseline in seven dimensions: sleeping, mental function, symptoms and discomfort, depression, distress, vitality and sexual activity. These differences in the 15D general score and different dimensions persisted for 12 months (Figure 5).



**Figure 5.** Mean 15D profiles at 12 months according to anxiety at the basal level.

## 6 DISCUSSION

### 6.1 RISK OF PRETERM BIRTH

Our studies show an increased risk for preterm birth after LEEP, and repeated LEEP increased the risk even further, almost three fold. The risk for preterm birth did not increase with increasing severity of CIN. The time interval between LEEP and subsequent birth did not have an effect on the risk for preterm birth. The risk for preterm birth seems to be associated specifically with treatment.

Various studies have reported the risk of preterm birth to be increased after local treatment for CIN (Sadler *et al.*, 2004; Albrechtsen *et al.*, 2008; Castanon *et al.*, 2014), and these are all in line with our results. Studies concentrating only on LEEP as a treatment modality also report an increased risk for preterm birth, again in line with our results (Jakobsson *et al.*, 2009; Noehr, Jensen, Frederiksen, Tabor and Susanne K Kjaer, 2009; Stout *et al.*, 2015).

Our study showed that repeated LEEP increased the risk of preterm birth almost threefold compared to the general population. This in accordance with other reports (Ortoft *et al.*, 2010; Kyrgiou *et al.*, 2016a). The 2017 Cochrane review reports that the risk of preterm birth in one treatment is increased 1.75 times versus no treatment, but after repeat treatments versus no treatment, it is increased more than threefold (Kyrgiou *et al.*, 2017). The increasing depth of the excised cone has been proven to increase the risk for preterm birth especially with cones deeper than 15 mm (Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009a; Kyrgiou *et al.*, 2016b), and this agrees with the findings of repeated LEEPs. On the other hand, cones of less than 10 mm in depth have been shown to have a limited effect on the risk for preterm birth (Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009a; Kyrgiou *et al.*, 2016b).

In our study, the risk for preterm birth did not increase with increasing severity of CIN. The risk for preterm birth also increased for women with LEEP and non-CIN lesions with milder HPV lesions. This finding is in accordance with other register-based studies from Denmark and Norway (Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009a; Bjørge *et al.*, 2016). However, the risk for preterm birth was increased over twofold for women with carcinoma *in situ* or carcinoma and was close to the risk caused by repeated procedures. This is most likely due to the more profound depth of the LEEP rather than the severity of the lesion, for deeper cones are undoubtedly performed for such lesions.

The time interval between LEEP and subsequent birth and the risk for preterm births are important for women planning pregnancies. We found that the time interval between LEEP and subsequent birth did not affect the

risk for preterm birth. Our results indicate that women do not have to postpone pregnancy after LEEP. This finding is supported by large register-based cohort studies from England and Norway (Castanon *et al.*, 2014; Bjørge *et al.*, 2016). An American study also found no effect on preterm birth with shorter intervals from LEEP to birth. However, they found that shorter intervals between treatment and pregnancy increased the risk for spontaneous first-trimester miscarriages (Conner *et al.*, 2013). A short (<12 months) interval between LEEP and pregnancy was also found by Ciavattini *et al.* (Ciavattini *et al.*, 2015) to increase the risk of spontaneous first-trimester miscarriages.

There have been questions regarding the effect of the comparison group used on the association reported between excisional treatment for CIN and preterm birth. External comparisons of the general population from registers has been suggested to over-inflate the risk associated. In our studies, we used multiple comparison groups: an external comparison to women in MBR, an internal comparison of births before and after LEEP for each woman, and women with disease but no treatment. We found the strongest association when using an external comparison, but one also existed when using other comparison groups. When we compared women with CIN1 and treatment to women with CIN1 and no treatment, however, the difference was not significant. Possibly more superficial cones were performed for the mild CIN1 lesion, which partly explains the insignificance. Most of the studies published have used external comparison groups (Noehr, Jensen, Frederiksen, Tabor and Susanne K Kjaer, 2009; Bjørge *et al.*, 2016), some have used internal comparisons (Albrechtsen *et al.*, 2008) and very few have used untreated CIN patients (Ortoft *et al.*, 2010). All of these studies supported our results. A meta-analysis concentrating on the comparison groups concluded that the risk for preterm birth was increased after excisional treatment, irrespective of comparison group (Kyrgiou *et al.*, 2016a).

Conflicting results have also been reported about the role of LEEP in the increase of risk for preterm birth. It has been suggested that this increase in risk is rather a consequence of CIN itself or other confounding factors present in women with CIN. In a study by Shanbhag *et al.*, the increased risk for preterm birth was increased in women with CIN3 compared to the general population, and no difference between treatment modalities (including no treatment) was found (Shanbhag *et al.*, 2009). However, the number of untreated women was very small compared (n= 87) to the excisional treatment group (n=1103), and no explanation was given as to why these precancerous lesions were left untreated. Poor compliance may be one reason for treatment, and these women may have other risk factors for preterm birth, e.g., socioeconomic factors, smoking, narcotics, poor education, etc. In a large register-based study from England, the risk for preterm birth was associated with excisional treatment of CIN but to a substantially lesser extent than reported in other studies, and the writers

highlighted the importance of confounding factors (Castanon *et al.*, 2012). One meta-analysis by Conner *et al.* also concluded that the increased risk for preterm birth is present for all women with CIN, irrespective of treatment (Conner *et al.*, 2014). Still, recent large meta-analyses and Cochrane reviews conclude that the risk for preterm birth is elevated after any excisional treatment. However, the data that associate CIN treatment and preterm births is, almost without exception, retrospective and lacking in randomized trials, and therefore, the results need to be interpreted with care (Kyrgiou *et al.*, 2016a, 2017). Changes in the vaginal microbiome, from less diverse to more diverse with HPV infection and CIN have also been reported (Ravel *et al.*, 2011; Lee *et al.*, 2013; Wiik *et al.*, 2019). Furthermore, CIN progression has been found to be associated with increasing diversity of the vaginal microbiome, and a trend toward association was found between a *Lactobacillus crispatus*-dominant microbiome and increasing severity of CIN (Mitra *et al.*, 2015). In another report comparing the microbiome in women with abnormal cervical cytology and that of women with normal cytology, the increasing diversity of the microbiome was associated with cervical abnormalities. They described a “risky microbiome” pattern to be paucity of *Lactobacillus crispatus* and dominance of *A. Vaginae*, *G. Vaginalis* and *Lactobacillus iners* (Oh *et al.*, 2015). Piyathilake *et al.* published a report contradicting the two previous ones; they found that a microbiome dominated by *L. iners* alone was associated with high-grade findings compared to a more diverse microbiome associated with low-grade findings (Piyathilake *et al.*, 2016). The role of the microbiome in association with CIN, LEEP and preterm birth needs further investigation.

All in all, the studies show that the risk for preterm birth is increased for women with CIN, and it further increases with treatment of CIN. Moreover, the risk increases directly with the increasing depth of the excision. Whether HPV infection, CIN and LEEP predispose women to an altered microbiome, thus partly explaining the increase risk for preterm birth, remains to be elucidated in future studies.

## 6.2 FOLLOW-UP AFTER TREATMENT

We found colposcopy alone at six months to perform poorly in predicting recurrent CIN. HrHPV alone seems to be an accurate and reliable test to predict cure and disease recurrence. Affected cone margins were associated with treatment failure, but alone, they were not a good predictor of treatment outcome. As a predictor of recurrence, cytology had a good specificity but poor sensitivity.

These results agree with previous studies (Bais *et al.*, 2009; Asciutto *et al.*, 2016; Arbyn *et al.*, 2017; Garutti *et al.*, 2017). Co-testing with hrHPV and cytology has been suggested as the follow-up method of choice (Asciutto *et al.*, 2016), and some reports show hrHPV testing alone to be superior



(Hormuzd A Katki *et al.*, 2013). In a report by Kocken *et al.*, the post-treatment risk of high-grade disease after negative co-tests at 6 and 24 months was at the same level as the general population with a negative screening result (Mariëlle Kocken *et al.*, 2011). However, Gosvig *et al.* found that after conization, the risk of HSIL in hrHPV-negative women was similar to women who were hrHPV- and cytology-negative. Moreover, the risk of recurrence for hrHPV-negative women in post-treatment was comparable to hrHPV-negative women in the general population, but for an unknown reason, this was true for only five years (Gosvig *et al.*, 2015). Also, a recent retrospective register-based study by Bruhn *et al.* found hrHPV alone to be a reliable test of cure after conization for HSIL lesions (Bruhn, Andersen and Hariri, 2018). A prospective study from Italy compared hrHPV and cytology in detecting women at risk for recurrent disease after treatment for HSIL. In a five-year follow-up, they found hrHPV testing to reliably predict treatment outcome with an NPV of 100% at five years. However, cytology alone was found to have similar results (Garutti *et al.*, 2017).

The role of colposcopy has been unclear in the CIN post-treatment follow-up. We found the performance of colposcopy at six months to predict treatment failure or cure to be poor. Previous studies on the issue are scarce. Old studies dating back a few decades have conflicting results on the necessity or advantages of colposcopy (Baldauf *et al.*, 1996; Flannelly *et al.*, 1997). In a report from Italy, Garutti *et al.* assessed the hrHPV, cytology and colposcopy at six months, but the thresholds for colposcopy positivity/negativity are not reported, nor the PPV and NVP for colposcopy (Garutti *et al.*, 2017). When considering the psychological burden of colposcopy described in the following chapter, based on these results, colposcopy should be omitted in the primary follow-up after treatment of CIN and reserved for those who are hrHPV-positive (co-testing).

Affected cone margins have been shown to be associated with treatment failure and are also suggested as a predictor of treatment outcome (Dobbs *et al.*, 2000; Ghaem-Maghani *et al.*, 2011b). However, a recent large meta-analysis stated that, even if margin status is associated with treatment failure, it does not predict efficiently treatment outcome, which agrees with our study. Alder *et al.* followed patients for 11–16 years for recurrent disease after conization for HSIL lesions. They found patients with affected margins to be at higher risk for recurrent disease, but the risk was increased, especially in those with affected endocervical margins (Alder *et al.*, 2019).

In our study in all women regardless of histopathological grade or reoperation 123 out of 491 (25.0%) women had affected margins, and 38 (26.8%) of those had a reoperation because of the margins. Almost half of the patients with positive endocervical margins had a reoperation in contrast to only 20% with affected ectocervical margins. Moreover, in only 26.3% of the reoperation cones, an HSIL lesion was still present. Of those with persistent HSIL at reoperation, most had affected endocervical margins at the original cone. Our findings imply that reoperations should not be performed for affected margins. Careful surveillance with hrHPV tests is an acceptable and

recommendable approach.

Traditionally, colposcopy has still been part of the post-treatment follow-up in many countries including Finland and, for example, Germany even though American and English guidelines recommend hrHPV as TOC. However, before there was little data on performance or value of colposcopy in follow-up. In light of already the preliminary results of this study, the follow-up guidelines at the Helsinki University Hospital Colposcopy Clinic were altered, and since spring 2019, colposcopy has been omitted in the follow-up at six months and replaced by an hrHPV and cytology co-test. The same update has been added to the Finnish Current Care guidelines.

## 6.3 HRQOL

### 6.3.1 HRQOL AND ANXIETY

We found that cytological abnormalities leading to colposcopy did not affect the general HRQoL. However, they seemed to be associated with anxiety and impaired psychological aspects of HRQoL. The severity of the cytological abnormality did not affect the results, suggesting that the knowledge of any abnormality causes the same psychological effects.

Our findings support older studies on the psychological effects of abnormal cytology (Bell *et al.*, 1995; Gray *et al.*, 2006). Maissi *et al.* found that general HRQoL was not affected six months after an abnormal cytology result, which is in line with our results (Maissi *et al.*, 2005). A Dutch study also supports our results, reporting that referral for colposcopy for abnormal cytology does not affect HRQoL in general, but mental health and anxiety scores were negatively affected (Korfage *et al.*, 2014).

Data on the effect of hrHPV on HRQoL and anxiety is also available. An English study compared anxiety in four different groups of women: women with borderline or mildly abnormal cytology who are positive or negative for HPV and women not tested for HPV with normal cytology or borderline or mildly abnormal cytology. Women with abnormal cytology and HPV positivity were more concerned, distressed and anxious than the other three groups. However, testing negative for HPV was not reassuring, as HPV-negative women with abnormal cytology were no less anxious than those not tested for HPV (Maissi *et al.*, 2004). The six-month follow-up results of this study showed no difference between the four groups in anxiety, distress or HRQoL. Levels of concern were highest at six months for women with an abnormal smear and not tested for HPV (Maissi *et al.*, 2005). In a Chinese study by Wang *et al.*, the psychological burden did not differ between women with abnormal cytology and positive HPV and those with abnormal cytology and no HPV testing (Wang *et al.*, 2011).

The length of the negative psychological effect is still unclear. A follow-up of the TOMBOLA trial found that women with low-grade cytology have

substantial initial psychological burden, irrespective of management (surveillance versus colposcopy) (Fielding *et al.*, 2017). We found that the psychological burden observed at baseline persisted for 12 months. This contradicts Maissi *et al.*, who found that the psychological effect was no longer present at 6 months (Maissi *et al.*, 2005). In the study by Hellsten *et al.*, the changes in psychological effects were found at baseline but not at any other follow-up time points during the next 24 months (Hellsten, Sjöström and Lindqvist, 2007). Korfage *et al.* also found the initial psychological effects to improve during the follow-up (Korfage *et al.*, 2014). The retrospective part of our study is in line with these results, showing that the patients' HRQoL does not differ from that of the general population eight years after diagnosis.

Results of the effect of lesion severity on the effect on HRQoL are conflicting. A Swedish study found the severity of histology to have no effect on results (Hellsten, Sjöström and Lindqvist, 2009). A Chinese study, in contrast, found women with CIN to have greater psychological burden than women with just cytological abnormalities (Wang *et al.*, 2011). In our study there was a small but statistically significant difference in the dimension of distress and vitality in dysplasia group compared to those with no dysplasia.

A report from the TOMBOLA trial found that women with pre-existing anxiety are at increased risk of anxiety in the follow-up. Moreover, worries about cervical cancer and fertility were significantly higher in women with HSIL lesions (Sharp *et al.*, 2015). In our study, women who had higher initial levels of anxiety measured by STAI at baseline had significantly lower HRQoL scores at baseline, 6 months and 12 months, suggesting that a tendency toward anxiety negatively affects HRQoL. This finding is supported by a Swedish study stating that depressive mood was a predictor of high State anxiety levels (Hellsten, Sjöström and Lindqvist, 2006). Sharp *et al.* investigated factors associated with psychological burden of colposcopy and found that younger age, CIN2/CIN3 and bleeding after colposcopy are predictors of distress (Sharp *et al.*, 2013).

A systematic review on the psychological effects of colposcopy states that the negative psychological effect exists, but the studies bear high rates of heterogeneity and call for more research (O'Connor *et al.*, 2016).

### **6.3.2 SEXUAL FUNCTION**

There is increasing awareness of adverse effects of LEEP on quality of life, especially sexual well-being, and it has been extensively discussed on social media. The very widely circulated American women's magazine, *Cosmopolitan*, published an article in April 2019 presenting women with problematic sexual lives after LEEP (Smothers, 2019). Various studies have concentrated on sexual function after LEEP; there seems to be an association with decreased sexual function, but only for a short duration (Inna,

Phianmongkhol and Charoenkwan, 2010; Serati *et al.*, 2010). However, in one longitudinal two-year study, the effect lasted for two years (Hellsten, Lindqvist and Sjöström, 2007). We found the sexual function dimension to be significantly lower in women who had undergone LEEP than in the general population eight years after dysplasia diagnosis. All previous studies concentrate on the psychological effects of sexual well-being, and no data have been found on the effects of LEEP on actual sexual functions (Hellsten, Lindqvist and Sjöström, 2007; Inna, Phianmongkhol and Charoenkwan, 2010; Serati *et al.*, 2010). However, clinicians should be aware of this aspect and should prepare for questions on sexuality.

### **6.3.3 INTERVENTIONS TO REDUCE ANXIETY DURING COLPOSCOPY**

Different interventions to reduce anxiety during colposcopy have been studied. In a Cochrane analysis, background music during colposcopy appeared to reduce anxiety (Galaal *et al.*, 2011); this would be an easy and cost-effective intervention to implement. Surprisingly, information leaflets did not reduce anxiety, but they did reduce psychosexual dysfunction (Galaal *et al.*, 2011). Also, pain perceived during colposcopy has been studied, and oral ibuprofen, topical benzocaine or xylocaine or topical benzocaine/xylocaine spray do not decrease pain sensation compared to placebo (Clifton, Shaughnessy and Andrews, 1998; Church, 2001; Öz *et al.*, 2015). In Finland, local anesthesia is not always used when obtaining biopsies in colposcopy, although it has been shown that local anesthetic injections before punch biopsies decreased pain (Kiviharju *et al.*, 2017). Indeed, local anesthesia decreased the number of women experiencing severe pain during colposcopy. It was effective in reducing pain also for endocervical curettage. This should also be taken into consideration in clinical practice.

## **6.4 STRENGTHS AND LIMITATIONS**

A strength of Studies I and II is the use of high-quality MBR and HDR data, enabling large study cohorts with the absence of recall, reporting and participation bias. This improves the credibility of our results. In both studies, adjustments for maternal age, socioeconomic status, marital status, urbanism and previous preterm birth further strengthens the results. We also included multiple comparison groups, which have not been used in many studies.

The risk of preterm birth has been shown to increase with increasing LEEP cone depth (Noehr, Jensen, Frederiksen, Tabor and Susanne K. Kjaer, 2009a; Kyrgiou *et al.*, 2016a). Depth of the excised cone is not available in

the registers, and thus, we did not have this information for the study, which is a considerable limitation. In Study II, we divided women into two groups according to whether or not they had been treated. It is possible that women who were treated immediately for CIN1 were considered to have more severe findings in colposcopy.

A strength of study III was its prospective and clinical setting. The generalizability of results is strong, given that the cohort is derived from an everyday clinical practice of a single referral center. Study III was limited by a lack of some follow-up data reflecting true clinical practice. Most recurrent cases occur within two years of CIN treatment, and the follow-up period of this study covers that. However, a longer follow-up period would be needed to rule out later disease recurrence. The number of immediate repeat operations was high in our study, and this might partly affect the overall recurrence results.

For Study IV, our strengths were that the patients came from an everyday clinical practice from the University Hospital Colposcopy Clinic and not from a selected group of patients. The use of a general HRQoL instrument instead of a disease-specific instrument or questionnaire gives us a larger perspective on the whole HRQoL of patients. Furthermore, the strengths also include the 12-month follow-up as well as the retrospective arm's insight into the prolonged effect on HRQoL.

A limitation of Study IV is that we have no data on the non-respondents, and a response bias cannot be ruled out, even though we have no reason to assume the non-respondents would suffer from lower HRQoL. Another limitation is our lack of knowledge on the patients' HPV status and other background characteristics to rule out all possible confounding factors.

## 6.5 FUTURE ASPECTS

Of the approximately 570 000 new cervical cancer cases, 85% occur in low- and middle-income countries, and 90% of deaths due to cervical cancer occur in the same area (Bray *et al.*, 2015; Ferley *et al.*, 2018). This is mostly due to poor or non-existent screening programs (Vaccarella *et al.*, 2013) or poor coverage of programs (Gakidou, Nordhagen and Obermeyer, 2008) but is also due to the high prevalence of HIV (Mukanyangezi *et al.*, 2019). Simultaneously, vaccination coverage in low-income countries lags far behind compared to high-income countries (Bruni *et al.*, 2016), even though geographic variations in vaccination coverage also occurs in high-income countries like Finland. The future challenge is how to provide adequate screening and primary prevention for women at highest risk for CIN and cervical cancer and how to decrease the incidence of cervical cancer worldwide.

Balancing between a good oncological outcome and acceptable adverse effects in the treatment of CIN is important. As LEEP has been associated with preterm birth, this should always be considered when choosing the procedure, but oncological safety should not be compromised (Kyrgiou *et al.*, 2016b; Lara-Peñaranda *et al.*, 2019). Good prospective data on the association of LEEP treatment and preterm birth are lacking, and due to the oncologic nature of CIN, randomized data are unexpected. In years to come, ongoing prospective trials will show the association between LEEP and preterm birth.

As screening (Ronco *et al.*, 2010) and post-treatment follow-up (Arbyn *et al.*, 2017) shift toward HPV testing, the need for new management and follow-up guidelines will become more urgent as persistent HPV positivity and normal cytology after treatment increases. In addition, regarding the recurrence, HPV persistence after treatment can represent either true persistent infections, recurrence of HPV or newly acquired HPV infections (Hoffman *et al.*, 2017). More research is needed to clarify the role of persistent HPV infection and for intervention guidelines for persistent HPV after treatment.

## 7 CONCLUSIONS

Based on this thesis, the following conclusions can be drawn:

1. In our studies, increasing severity of the CIN lesions did not increase the risk for preterm birth. Short time intervals between LEEP and subsequent birth did not predispose to preterm birth, suggesting that women do not have to postpone pregnancy after LEEP.
2. The association between LEEP treatment and preterm birth was confirmed in our studies; it was shown that it is not only the existence of CIN lesions that increases the risk for preterm birth but also the treatment itself.
3. Colposcopy performed poorly as a predictor of treatment outcome in the follow-up after treatment of CIN and can therefore be omitted in the primary follow-up. HrHPV proved to be a reliable tool in the follow-up after treatment.
4. Our study confirms the psychological burden in women with abnormal cytology referred to colposcopy. The negative mental effect is not dependent on the severity of the abnormal finding and, hence, it seems that the presence of any lesion causes anxiety. The general health-related quality of life was unaffected in our study.

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